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THE RAT IS FAT
(See Page 275)

Leopard Frogs

Pigmented Platyfish

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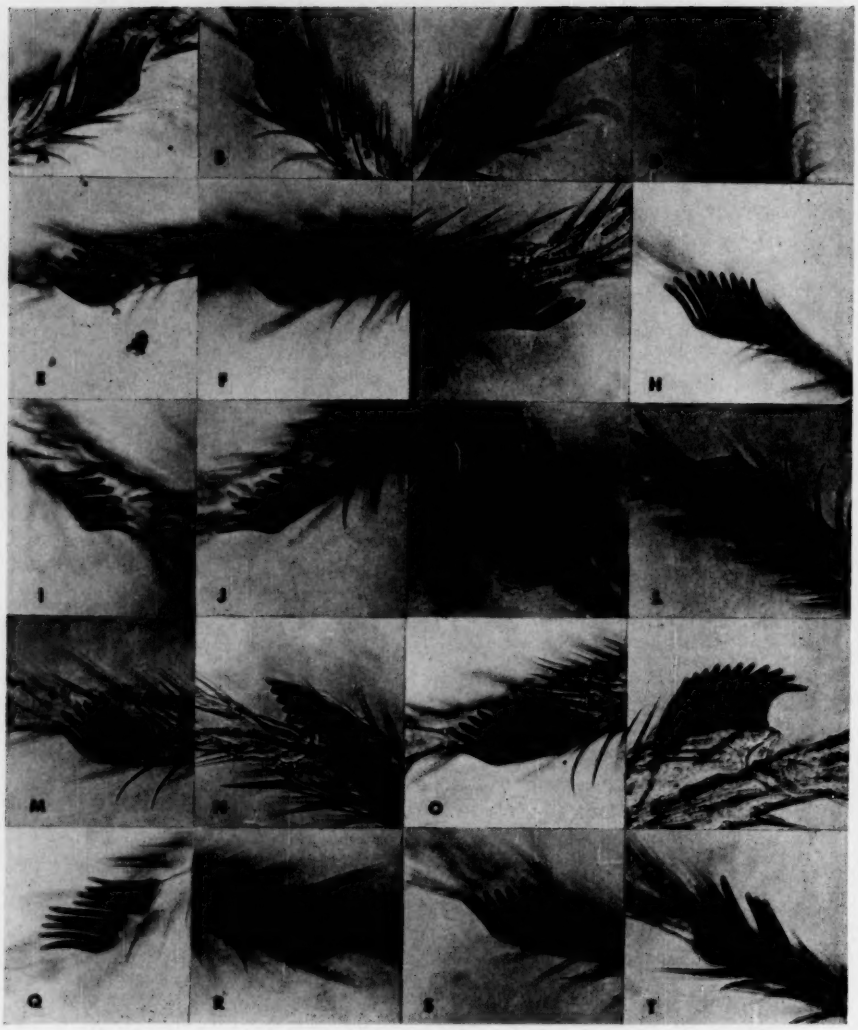


SEX COMBS

Figure 1

Photographs of sex combs of third chromosome aneuploids, unbalanced male forms, and ordinary diploid males. *A* and *H*—left and right combs of 2-6 hypo-diploid male; *B*—*H7L* hyper-diploid male; *C* and *D*—left and right combs of *H7L* hyper-intersex; *E*—*H7-13* hypo-diploid male; *F*—*H7-13* hypo-intersex; *G*—*85CR* hyper-intersex; *I* and *J*—left and right combs of *2R* hyper-diploid male; *K*—*12L* hyper-diploid male; *L* and *M*—*12L* hyper-intersex; *N*—*85CL* hyper-intersex; *O* and *P*—left and right combs of normal diploid male; *Q*—super-male; *R* and *S*—left and right combs of control *2X3A* intersex; *T*—right comb of control *2X3A* intersex.

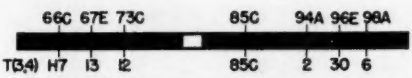
SEX COMBS IN UNBALANCED MALE FORMS OF DROSOPHILA MELANOGASTER

SARAH BEDICHEK PIPKIN*

SINCE studies of the terminalia of neither second nor third chromosome triploid aneuploids revealed a definite qualitative sex shift[15,16,17], the sex combs of certain male or intersex aneuploids have been investigated. This work was undertaken to compare the sex combs of hyper-intersexes carrying in excess of *2X3A* various fragments of chromosome 3 with their sibling *2X3A* intersexes to learn if a modification toward maleness occurred in the former. Sex combs of males hypo-diploid for a short section of chromosome 3 were examined to see if a change in the female direction had taken place. Sex combs of hyper-diploid males and super-males were studied since these represent unbalanced male forms where no sex shift is expected.

Materials and Methods

The 3,4 translocations used and methods of obtaining the triploid aneuploids have been described elsewhere[16,17]. A diagram of the third chromosome with the salivary map points of breakage of the translocations used is given in Figure 2. Either the left-hand fragment (L) or the right-hand fragment (R) of the respective translocations + *2X3A* was present in a given hyper-intersex. Figure 3*A* shows the genetic composition of a hyper-intersex and of its sibling control *2X3A* intersex. Sex combs of the following long-region hyper-intersexes were examined: *12L* + *2X3A*, *85CL* + *2X3A*, *85CR* + *2X3A*, *89ER* + *2X3A*. Short-region hyper-intersexes studied included *H7L* + *2X3A*, *2R* + *2X3A*, *30R* + *2X3A*, and *H6R* + *2X3A*. Sex combs



SALIVARY MAP OF CHROMOSOME 3

Figure 2

Diagram of the salivary map of chromosome 3, showing below, the points of breakage of 3,4 translocations, and above, the corresponding locations on the salivary map.

of *30R* + *2X3A* and *2R* + *2X3A* hyper-intersexes were of interest because their mean sex type (scored according to the external genitalia) was slightly but significantly more male than the mean sex type of their respective *2X3A* intersex siblings[16]. The genetic composition of hyper-diploid and hypo-diploid males, respectively, is shown in Figure 3*B* and *C*. Sex combs of hyper-diploid males carrying one of the following fragments in excess of *1X2A* were studied: *2R*, *H7L*, and *12L*. Sex combs of hypo-diploid males with the section between *T*(3,4) *A2* and *T*(3,4) *H6* haploid and also those with the section between *T*(3,4) *H7* and *T*(3,4) *A13* haploid were examined. The latter hypo-ploid males were chosen for study because approximately one-fourth possessed rotated genitalia. The region *H7-13* is very nearly the same region studied by Patterson *et al.*[12], who reported hypo-diploid males with frequently rotated and imperfect genitalia.

Super-males and control normal diploid males here studied were taken from the y^2; *ru ca* triploid stock used in third chromosome aneuploid studies.

Whole forelegs of flies were stored in 70 percent alcohol and mounted either in euparol or clarite. Right legs were cut shorter than left so that these could

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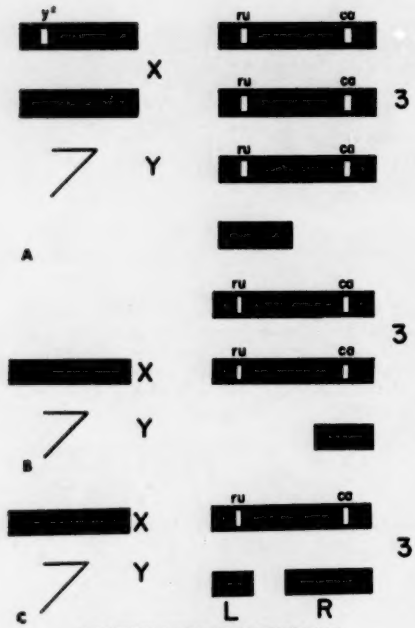

GENETIC COMPOSITIONS

Figure 3

A—Genetic composition with respect to X and third chromosomes of a hyper-intersex; *B*—Genetic composition with respect to X, Y, and third chromosomes of a hyper-diploid male; *C*—Genetic composition with respect to X, Y, and third chromosomes of a hypo-diploid male.

be distinguished on the slide when the sex comb prongs were counted. Where possible, sex combs of 25 individuals of each genotype were examined.

Results

Photographs of sex combs of certain aneuploids, the normal diploid male (*1X2A*), the super-male (*1X3A*), and the ordinary *2X3A* intersexes are presented in Figure 1. Sex combs of the unbalanced male forms (aneuploids, super-male, and *2X3A* intersexes) differ from the sex combs of the normal diploid male by a reduction in prong number; presence of occasional or, in some instances, rare gaps in the comb (divided combs); and, in the case of the super-male, an increase in the asymmetry of prong number in right and left combs of the same individual. The sex comb teeth of diploid males are pressed close together, and the comb possesses a characteristic curve not usually seen in combs of intersexes and aneuploids. In the latter, the teeth are spread apart and appear more stubby and thick than the teeth of the normal male comb, though Hannah-Alava and Stern[11], from actual measurements of intersex and diploid male combs, found no difference in thickness of corresponding prongs.

A comparison of mean tooth number in the sex comb of the control diploid male and in those of the various chromosomally unbalanced male forms is given in Table I. Here it is of interest that the mean tooth number in hyper-intersexes carrying a short right-hand end fragment of chromosome 3 (*i.e.*, *2R*, *30R*, or *H6R*) does not differ significantly from the mean tooth number of sex combs of the corresponding *2X3A* sibling control intersexes. All chromosomally unbalanced male forms have fewer teeth in the sex comb than the control diploid male. The degree of reduction of tooth number is correlated with the degree of chromosomal imbalance. Super-males (*1X3A*) have the fewest sex comb prongs and the greatest qualitative disturbance of the comb. Hyper-intersexes with longer fragments usually showed greater reduction in tooth number than hyper-intersexes with shorter fragments. Gaps in the sex comb were extremely rare in ordinary *2X3A* intersexes but were found in hyper-intersexes *89EL*, *H7L* (Figure

TABLE I. Mean tooth number of the sex combs of hyper-intersexes, their corresponding control sibling *2X3A* intersexes, normal diploid males, hyper-diploid males, hypo-diploid males, and super-males

HYPER-INTERSEX		CONTROL INTERSEX (2×3A)	
12L	6.50 ± 0.17		7.58 ± 0.16
85CL	6.09 ± 0.16		6.99 ± 0.14
2R	7.50 ± 0.24		7.40 ± 0.15
30R	8.00 ± 0.23		7.58 ± 0.22
6R	8.12 ± 0.23		8.00 ± 0.21
H7L	(5–6) (2 flies)		
89EL	(7,6) (1 fly)		
89ER	(7,10) (1 fly)		
HYPO-DIPLOID ♂		CONTROL DIPLOID ♂ (1×2A)	
H7–13	8.57 ± 0.14		10.84 ± 0.14
2–6	(9,9) (1♂)		
HYPER-DIPLOID ♂		SUPER-♂ (1×3A)	
2R	9.27 ± 0.11		8.56 ± 0.22
12L	(7,7) (1♂)		
H7L	(6–9) (3♂)		

1*D*); hypo-intersexes *H7-13* (Figure 1*F*); hypo-diploid males *2-6* and *H7-13*; and most frequently, in super-males, where seven of 40 combs studied were of this type. The gap in a divided comb may occur at different places in the comb. The teeth in proximal and distal combs numbered 3,4; 4,4; 6,2; 5,3; 4,3; 8,2; 7,8, respectively, in seven super-males showing divided sex combs. In the last case, an additional prong was present on the extensor surface of the tarsus near prong 5. The presence of a prong "out of line" with the rest of the comb was also observed in the hyper-intersex *89EL*.

The asymmetry of sex comb prong number characteristic of both normal diploid males and of ordinary *2X3A* intersexes is likewise present in the aneuploids' sex combs and is actually more pronounced in the super-male sex combs. Of 26 normal diploid males, 16 showed differences in prong number between right and left legs of the same individual, the difference ranging from one to three prongs. In ordinary *2X3A* intersexes, 51 of 82 examined displayed such asymmetry. In super-males, 15 of 20 combs studied were asymmetrical.

Discussion

A reduction in sex comb tooth number indicating a shift from the 8-pronged intersexual comb in the female direction has been found by Pipkin[13] in the case of certain X-chromosome triploid aneuploids which have rudimentary combs of one or two prongs. On the other hand, an increase in comb teeth from four to six in triploid females carrying the hermaphrodite gene, *Hr*, in one third chromosome to 10 to 12 teeth in sex combs of triploid females homozygous for its allele, *tra*, studied by Gowen and Fung[9], demonstrates an obvious modification in the male direction, using the sex comb tooth number as a criterion of the degree of sex shift.

No rudimentary sex combs were found on hyper-triploid females carrying *3X3A* plus short or long fragments either of chromosome 2[15] or of chromosome 3[16,17], although Bridges[1] reported that individuals with *3X4A* (determined cytologically) were similar to *2X3A* intersexes. According to external genitalia studies, no sexual shifts were observed in any of the surviving hypo-intersexes involving sections of chromosome 2[15] or of chromosome 3[16,17]. It was therefore concluded that male-determining factors present both in chromosome 2 and in chromosome 3 were responsible for the shift toward maleness in ordinary *2X3A* intersexes[17]. Hyper-intersexes bearing either of two short right-hand end regions of chromosome 3 showed mean sex types (classed according to the external genital ducts) slightly more male than those of sibling control intersexes[16], but the mean sex comb tooth number of these hyper-intersexes did not differ significantly from the mean sex comb tooth number of sibling control intersexes. Furthermore, the hyper-intersexes with longer fragments showed the greater reduction in number of sex comb teeth. Hypo-diploid males did possess fewer sex comb teeth than control diploid males, but this reduction is not taken as a shift toward femaleness but rather as the result of a growth disturbance caused by aneuploidy, A lesser reduction in mean sex comb tooth number was likewise found in hyper-diploid males, and a more pronounced reduction in super-males, where no sex shifts are expected. Thus the studies of sex combs and of external genital ducts of third chromosome hyper-intersexes are in agreement in failing to indicate a definite shift toward maleness.

On the other hand, it may be that no increase in sex comb tooth number should be expected in autosomal hyper-intersexes. To anticipate such an increase supposes that the reduction in ordinary *2X3A* intersex sex combs, as compared with the tooth number in diploid males, is due to intermediate sexual development, an inhibition of the development of the comb owing to female development within the same individual. It may be argued that the sex combs of triploid intersexes represent a complete male development, the small reduction in prong number being due to a growth disturbance dependent upon the unbalanced *2X3A* genotype but not upon a feminizing (hormonal) influence. The following are some reasons for thinking that triploid intersexes possess a complete male development in the early differentiated sex comb (with the exception of the very rare, extreme female-type intersexes which lack combs): (1) Dobzhansky[2] found that selection of a triploid line yielding male-type intersexes was more effective and proceeded more rapidly than selection of a triploid line yielding female-type intersexes. (2) Although extremely rare type VI *2X3A* intersexes lacking sex combs and occurring in two to three percent of the highest selected female-type intersex producing line were described by Dobzhansky and Bridges[3], Pipkin[13] found that the mean prong number of sex combs of triploid intersexes of the extreme male type I did not differ significantly from the mean sex comb prong number of intersexes of the female type IV (with imperfect female genitalia, sex combs). Hannah-Alava and Stern[11] reported a similar finding for sex combs of male-like, intermediate, and female-like triploid intersexes. (3) Similarly, Hannah[10] found that Strapped, a digenic male-limited character, was expressed in both male- and female-type triploid intersexes. (4) Pipkin[16] found the variation in total time of development of male-type and female-type triploid intersexes to be the same. Furthermore, Gowen and Fung[9] stated that the triploid intersex genotype,

2X3A, is little affected by *Hr*, the hermaphrodite gene, and *tra*, its recessive allele, both of which strongly modify development in the male direction in diploid and triploid females, but fail to affect the diploid male.

Another case in which it is possible that a growth disturbance accounts for the reduction of mean sex comb tooth number is the finding by Stern and Hannah[19] that sex combs of mosaic legs or segments of legs of gynandromorphs averaged 1.3 prongs fewer than those of their diploid male siblings, these authors concluding, "whether this influence derives from a quantitatively specific nature of the female tissue, transmitted to the male tissues in a hormonal way, or whether the quantitative differences in general metabolism and duration of development which characterise the two normal sexes are instrumental, must remain an open issue."

Recognizing the predominantly male-like character of *Drosophila* triploid intersexes, Goldschmidt[7] described them as "male" intersexes in contrast with *2X2A* diploid intersexes homozygous for an intersex mutant which he termed "female" intersexes. He considered *2X3A* triploid intersexes to be sex mosaics in time, male development preceding female development, a view supported by the work of Dobzhansky and Bridges[3]. Although contested and defended, this theory emphasizes the essentially male nature of most triploid intersexes in *Drosophila*.

Four mechanisms have been found which modify the sexual development of ordinary *2X3A* intersexes: (1) Dobzhansky[2], working with a triploid line producing intersexes ranging from sex types I to V, found that culturing intersexes at 20° C. favored a shift toward maleness; at 28° C. a shift toward femaleness. (2) Dobzhansky[2] located a particular third chromosome which in one dose shifted the mean sex type of intersexes in the female direction. (3) Fung and Gowen[5] reported female-type triploid intersexes to have higher numbers of fourth chromosomes than intermediate and male-type intersexes. (4) In one cross, Dobzhansky and Schultz[4] found that the mean sex type of intersexes arising from *1X2A* eggs does not differ from the mean sex type of intersexes arising from *2X2A* eggs. The former intersexes lack a Y chromosome; the latter possess one. These authors concluded that the Y chromosome does not influence the sexual development of intersexes. Pipkin[14] crossed males of 20 different wild strains to an inbred y^2 *v f* (yellow-2, vermillion, forked) triploid line. In six of these crosses, the wild-type intersexes, coming from *1X2A* eggs, were significantly more male-like than the intersexes displaying the recessive marker mutants y^2 *v f*, arising from *2X2A* eggs. In the remaining 14 crosses, the frequencies of sex types of wild-type and y^2 *v f* intersexes were homogeneous (did not differ significantly). The author concluded that these variations in sex types of the two kinds of intersexes were owing to dominant modifier(s) in some of the wild-type X chromosomes, although the possibility of modifiers in the Y chromosome was not completely ruled out. Further crosses of wild-type males to triploids homozygous for the mutant y^2 (yellow-2), carried out in connection with other experiments, have confirmed the observation that, in a number of crosses, the yellow-2 intersexes (from *2X2A* eggs) are significantly more female-like than the wild-type intersexes (from *1X2A* eggs) (Pipkin, unpublished). Other explanations than the one given by Pipkin[14] may account for these data. Possibly intersexes arising from *1X2A* eggs are sometimes more male-like than their sibling intersexes arising from *2X2A* eggs because of the action in the *1X2A* eggs before fertilization of autosomal male-determining genes which have twice the dosage of X chromosome genes in these eggs. This explanation was applied by Goldschmidt[6] to some results found in his Bd-Mn intersexes. He further pointed out that a case of diploid intersexuality has been studied by Sturtevant[20] in which male-determining genes acted in the egg before fertilization. Since the frequency of *1X2A* eggs is much higher than the frequency of *2X2A* eggs, this explanation would partially account for the preponderance of male-type intersexes in most triploid lines. However, intersex-triploid mosaics[8,18] prove that the decision of sex determination comes after fertilization as in the case of diploid males and females. Finally, the yellow intersexes arising from *2X2A* eggs may be more female-like in some cases because there may be a tendency of fourth chromosomes to segregate into the *2X2A* eggs with a higher frequency than into the *1X2A* eggs.

Summary

Sex combs of hyper-intersexes of the genotype *2X3A* with a fragment of chromosome 3 possessed no more prongs than their control sibling *2X3A* intersexes. An examination of sex combs of other unbalanced male forms, hyper-diploid males and hypo-diploid males, the aneuploidy involving sections of chromosome 3, and supermales (*1X3A*), revealed a slight reduction in sex comb prong number as compared with ordinary diploid males. It is suggested that the *2X3A* intersex sex comb in all but the extreme female-type intersex represents a complete male development. The reduction in prong number is thought to be due to a growth disturbance caused by the unbalanced genotype. Some factors influencing the degree of sexuality of *2X3A* intersexes are discussed.

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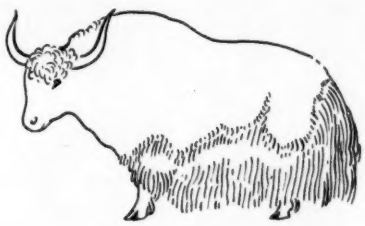

A DICTIONARY OF GENETICS AND CYTOGENETICS

ALTHOUGH four years old, this book* remains the most up-to-date and comprehensive in the field. Until 1958 the only serious attempt to compile a dictionary of genetics was that of R. L. Knight (reviewed in *Jour. Hered.* 40: 131, 1948). Of course, the unabridged dictionaries such as Webster's covered many technical terms, but not adequately for research workers. Rieger and Michaelis satisfy most of our needs: both English and German terms; explanations rather than definitions; a number of excellent diagrammatic illustrations; dates and literature sources; and abundant cross-indexing.

In fact, the book is practically an encyclopedia. Not merely words but also a huge number of phrases are gathered, such as "isolation estimate," "recombination index," and "UV-restauration." The bibliography at the end of the book is over 50 pages, comprising about 1500 references. The burgeoning microbial-genetics field has been carefully sifted.

The authors are on the staff of the Institute for Research on Cultivated Plants at Gatersleben (a branch of the Berlin-German Academy of Sciences). One might well wonder whether they could really cover the literature in English as well as in German, and

**Genetisches und cytogenetisches Wörterbuch.* (Second, enlarged edition.) R. Rieger and A. Michaelis. Springer-Verlag. Berlin. 648 pp., 8°, 149 illus. 1958. D M 49.60.

whether the animal-genetics side would receive sufficient attention. Of course, in a labor of such scope one must expect omissions and unevenness. I would like to call attention to some of these faults.

In the first place, while the authors have attempted to incorporate obsolete terms for their historic interest, many have been missed, at least from the literature in English. Examples are homoögonous, homotyposis, mnemon, poecilodynamous, schizogonous, and teleone.

Quantitative genetics (as distinguished from population) is not covered as such; filial regression is briefly defined, but there is no mention of Galton's laws. The terms midparent, diallel cross, mass selection, path coefficients, twin analysis, and heritability estimation are absent; "epistacy" is included, but without any hint that it has been confused with "epistasis."

Unlike Knight's work, the present treatise contains nothing on internationally established rules of nomenclature and symbols. While such rules are commonly ignored by many geneticists, they are not *ipso facto* to be ignored. Rieger and Michaelis merely give a few symbols, such as In, T, and M as used in *Drosophila*. The + symbol is introduced casually in connection with "normal allele," and under the heading "Standardtyp," which is well defined, but limited to genotype. There is no cross reference to wild type, which is defined as a phenotype. Antiquated symbolism is given under "duplicate genes," with no cross references to or between genocopy, mimics, polygenes, multiple factors, or factors, additive.

Incoherence is manifest in many other places also. For example, there is no cross reference between "dominant" and "codominant," between "heterosis" and "euheterosis," between "step allelomorph" and "pseudoallele," "komplex loci," "cistron," etc., or between "one-gene-one-enzyme hypothesis" and "Genwirkung" (gene action—occupying two and one-half pages). "Vererbung" is well defined, "heredity" is not (and no cross reference).

There are surprising omissions of relatively new terms, even some in titles of research papers, such as paramutation, genetic load, somatic segregation, bipaternity, and breakage-fusion-bridge cycle. Even more surprising is omission of many terms used by breeders: linebreeding, grading up, rotational and crisscrossing, prepotency, stud, pure, fix, blood fractions, telegony, split, half-sider, sire index, etc.

But again, these faults are minor, and it is remarkable that they are so few. Even though "geneticist" and "cytogeneticist" are not included, every such scientist should have ready access to the book. This work will serve as a basic reference for decades to come.

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GENETICS ON THE POPULATION LEVEL

THIS brief book* is intended to be a collection of theorems and established principles of population dynamics presented in compressed form. It may prove advantageous to the student as a kind of "college outline" of the subject; it will be useful to teachers also as a handy reference to the founding principles: gene and zygote frequencies in panmictic populations, forces acting on the gene pool, effects of mating systems, quantitative characters, and an all too brief discussion of "practical difficulties encountered" (ionizing radiation and selection for quantitative characters).

Since discussion is so limited, the student cannot hope to foresee the complexities in a real population. Stress has been placed on algebraic relationships with some numerical illustrations, especially in quantitative problems of interest to students of breeding. Many examples are clearly presented but they tend to be borrowed from more well known texts; and formulas are often applied without derivation or sufficient explanation for complete understanding.

To be a useful science, population genetics must be a synthesis of intelligent observation coupled with mathematical theory. The concrete details of natural population phenomena could hardly have been anticipated by the mathematician. There are so many well established principles of population genetics discovered by experimental analysis (e.g., the dependency of relative fitness upon epistatic interaction in relationally balanced systems and the integrating properties of populations) which have been barely touched upon or not discussed that perhaps the book should have been titled "Introduction to the Basic Dynamics of Genes in Populations."

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**Genetics on the Population Level.* Marianne Rasmuson. Scandinavian University Books (Svenska Bokförlaget). Stockholm. 192 pp. 1961.

A GENETIC ANALYSIS OF THE "EYESPOT" MUTATION OF TRIBOLIUM CONFUSUM

DANIEL J. MCDONALD AND NANCY JANE PEER*

SEVERAL mutations are known which affect the eye structure of flour beetles, *Tribolium castaneum* and *Tribolium confusum*. In *T. castaneum*, the autosomal, allelic mutations pearl (Park[4]) and pink, as well as the sex-linked mutation red, have been found, and this last gene, along with four others, has been used to map the X-chromosome of *T. castaneum* (Sokoloff *et al.*[6]). In *T. confusum*, Graham[1] described an autosomal mutation, pearl, which is phenotypically similar to the pearl of *T. castaneum*. The solid black eye color, found in laboratory strains as well as in naturally occurring populations, is considered to be the usual or wild-type condition, and there has been no indication that populations of *Tribolium* might be polymorphic for eye mutants.

This paper describes a new sex-linked eye mutation of *T. confusum*, eyespot (*es*), provides a measure of linkage between *es* and the lethal Striped (*St*) (McDonald[2]), and explores the possibility that some *T. confusum* populations may be polymorphic for *es* as well as for other mutations affecting the structure of the eye.

Methods and Results

The *es* mutation was first noticed in a wild-type strain of *T. confusum* which had originated from a single prefertilized female captured in a mouse feed bin at Columbia University in 1955. Since approximately 18 percent of the adults in this strain (designated strain 1) proved to be phenotypically mutant, a pure *es* strain was easily obtained. The mutation is characterized by a clear spot in the center of the eye which can be readily seen in young adults if a strong beam of light is passed through the insect's head. It becomes increasingly difficult to see as the insect ages, but never disappears completely.

Preliminary crosses with wild-type beetles from strain 1 suggested that *es* was a sex-linked recessive mutation, but the results were confused by the presence of heterozygotes among the strain 1 wild-type females chosen for the crosses. By selection and testing, a wild-type strain free of the mutation was obtained, and the results of crossing it with the *es* strain are given in Table I. It is clear that *es* is a sex-linked recessive with no obvious effect on the viability of either males or females.

Attempts to establish the linkage relationship between *es* and *St*, a recessive sex-linked lethal with a dominant phenotypic effect (McDonald[2]), were unsuccessful at first, for the *St* strain also proved to be contaminated with the *es* gene. This was not surprising, however, because the *St* strain was originally de-

TABLE I. Offspring obtained from crosses of the eyespot mutant with wild type

Parents	Mutant ♀ × Wild ♂				Mutant ♂ × Wild ♀			
Offspring	Wild type		Mutant		Wild type		Mutant	
	♂	♀	♂	♀	♂	♀	♂	♀
	0	389	385	0	230	270	0	0

*Department of Biology, Dickinson College, Carlisle, Pennsylvania. This investigation was supported by research grant RG-5836 (R1) from the National Institutes of Health, Public Health Service. The authors wish to express their appreciation to Miss Irene Tar and Mrs. Cynthia Wear for their technical assistance.

rived, in part, from strain 1. When females heterozygous for *St* and for the wild-type allele of *es* (*es*$^+$ *St*/*es St*$^+$) were finally derived and crossed with *es St*$^+$ males, consistent results were readily obtained. The data in Table II demonstrate that the genes *St* and *es* are about 37.6 crossover units apart on the X-chromosome. The smaller number of *St* heterozygotes among the female offspring is probably the result of the *St* gene's effect on viability, and has been encountered in other experiments. Most of the males carrying *St* are eliminated before the tenth day of the larval stage by the lethal action of the mutation (McDonald and Peer[3]), and hence, cannot be included in the linkage calculations. In the present experiments, 253 of these males survived, 167 *es*$^+$ *St* and 86 *es St*, and it is interesting to note that the numbers of the two kinds of *St* males are significantly different from the equal numbers expected (chi square = 24.95, $P < .001$). This may mean that *St* and *es* in some way interact in males, depressing viability still further.

Thirteen other strains immediately available were surveyed to determine if *es* might be a common allelomorph in *T. confusum* populations. One strain, started from a prefertilized female collected at the same time and place as the original female of strain 1, and three other strains, whose relationships to strain 1 are probably remote, were found to contain eye variants similar to *es*. Further information concerning these strains will be found in Table III.

Proceeding under the assumption that these variants are genetic, preliminary experiments were performed with two of the strains to explore the possibility that the variants might be allelic to *es*. The results of pair matings involving mutant males from strains 10 and 21 with virgin *es* females are given in Table IV. All male offspring are hemizygous for *es* and have eyespots as expected, but there are clearly three different situations among the female offspring. The F_1 of cross number 1, containing both mutant and wild-type females, could be obtained if the eye mutation under investigation was determined by an autosomal dominant gene introduced into the cross by a hetero-

TABLE III. Strains of *Tribolium confusum* containing mutants similar to eyespot

Strain number	Source	Percent mutants
1	Columbia University	18
6	Columbia University	2
17	University of Chicago	3
10	Forest Insect Laboratory, Sault Ste. Marie, Canada	44
21	McGill University, Montreal, Canada	4

TABLE II. Number and percentage of crossover and non-crossover offspring obtained from *es*$^+$ *St*/ *es St*$^+$ ♀♀ × *es St*$^+$ ♂♂ parents. The male and female data have been analyzed separately

	Female offspring						Male offspring*			
	Non-crossovers			Crossovers			Non-crossovers		Crossovers	
Run	*es*$^+$ *St* / *es St*$^+$	*es St*$^+$ / *es St*$^+$	%	*es St* / *es St*$^+$	*es*$^+$ *St*$^+$ / *es St*$^+$	%	*es St*$^+$	%	*es*$^+$ *St*$^+$	%
1	138	145	60.2	81	106	39.8	158	60.5	103	39.5
2	123	148	63.4	78	78	36.5	124	61.3	78	38.6
3	372	392	63.6	202	235	36.4	351	60.3	231	39.7
4	492	493	63.7	277	283	36.3	516	65.3	274	34.7
5	505	487	62.3	297	303	37.7	512	60.2	338	39.7
Total	1630	1665	62.9	935	1005	37.1	1661	61.9	1024	38.1

* Males carrying the *St* gene are encountered only infrequently and have, therefore, been excluded from the table.

zygous male. The data obtained from crosses 9 and 12, where the F_1 females are all mutant, may indicate the presence of an eye mutation allelic to *es,* but as there are other possible interpretations, further investigation is necessary. Finally, all the remaining crosses produced F_1 generations with wild-type females only. This result would be obtained if the new mutation depended upon a recessive non-allelic gene. The data suggest, therefore, that at least two other similar eye mutations besides *es* occur in *T. confusum* populations, and reveal a genetic diversity which hitherto seems to have escaped notice. In view of the surprising complexity of the situation, further analysis will be deferred to a later report.

Discussion

The origin of the *es* mutation in strain 1 is not known, but it must either have appeared by spontaneous mutation following the origination of the strain, or have been introduced by the prefertilized female used to start the strain. The latter possibility seems more probable and, if the phenotypically similar form found in strain 6 proves to be the *es* mutant, it would seem likely that *es* was a rather common allelomorph in the wild population from which strains 1 and 6 were taken. Whatever the origin, *es,* and perhaps the other eye mutations as well, seem to remain at fairly high frequencies in laboratory populations of *T. confusum.*

This also seems to hold true for the pearl mutation of *T. confusum,* for Graham[1] reports this mutation to have been observed in *T. confusum* stocks several years prior to his investigations, and at the time of his experiments pearl mutants constituted 22.8 percent of the populations.

The presence of two or more forms in a population at frequencies higher than might be expected from mutation alone suggests the existence of a balanced polymorphism. Sheppard[6], in a recent review of this subject, points out that a balanced polymorphism will usually depend upon an equilibrium of the selective forces involved, rather than upon the adaptive neutrality of the various phenotypes. While it is not clear at the moment which, if either, explanation applies to pearl and eyespot, there appears to be no strong selection against them. They seem, therefore, rather different from eye mutations found in *Drosophila,* many of which are adaptively disadvantageous. Possibly mutations which affect the eye and perhaps impair vision have less serious consequences for a flour beetle, which is essentially a flightless, burrowing organism adapted to a dark environment. Thus, unless the eye mutations produce other adverse effects, they may approach more closely a condition of neutrality.

Summary

The mutation "eyespot" of *T. confusum,* which produces a transparent area in the center of the eye, is due to a sex-linked recessive gene located 37.6 crossover units from the lethal Striped. The high frequency of the mutation in the laboratory population in which

TABLE IV. Offspring from the crosses of strain 10 and strain 21 males with eyespot females

Parents	Cross no.	Offspring			
		Mutant ♀	Wild ♀	Mutant ♂	Wild ♂
	1	9	26	36	0
	2	0	18	12	0
	3	0	27	15	0
Strain 10 ♂♂	4	0	30	24	0
es ♀♀	5	0	11	15	0
	6	0	29	29	0
	7	0	12	10	0
	8	0	6	9	0
	9	13	0	24	0
	10	0	28	19	0
	11	0	37	39	0
Strain 21 ♂♂	12	24	0	25	0
es ♀♀	13	0	27	31	0
	14	0	25	21	0
	15	0	28	24	0
	16	0	14	23	0

it was found suggests the existence of a balanced polymorphism. A survey of several other related and unrelated *T. confusum* strains has revealed the presence of other possible eye mutations in unusually high frequencies. It is suggested that if, in *Tribolium*, the eye is an organ of lesser importance, mutations affecting it may be more readily maintained in populations.

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Pigmentation—Physical and Chemical

HARVARD Medical School scientists, working at the Massachusetts General Hospital, have isolated the tiny cell structures which manufacture body pigments, and they have made them function in test tubes.

This basic achievement opens the way to physical and chemical studies of the secrets of racial coloration, benign conditions from freckling to baldness and a dozen disease states in which pigmentation is a factor. Many of the chemical mechanisms responsible for the conditions are now being studied at Harvard.

This was contained in a recent report by the American Cancer Society on pigment-cell research by Dr. Thomas B. Fitzpatrick, Professor of Dermatology, Dr. Makoto Seiji, a dermatologist from Japan, and Mr. M.S.C. Birbeck of the Chester Beatty Institute of London who is now temporarily at Harvard.

The group, by chemical and physical means, have obtained in pure form the color-producing particles, or organelles, and with them have learned how the natural animal and human pigment, called melanin, is synthesized.

Body coloring matter, melanin, is manufactured by specialized cells called melanocytes. In the skin of the average adult there are about 2,000,000,000 melanocytes strewn diffusely among ordinary skin cells. Melanocytes also are scattered among eye, hair and brain. The melanin they produce is taken up by other cells, and it gives them their color.

Melanin serves as a light-absorbing umbrella for an otherwise colorless body. Were it not for melanin, the sun would ultimately kill animals and humans living in sunny climates. Among the lethal effects is skin cancer, which is rare among dark people and common among light-skinned people.

Various races have precisely the same kind and number of melanocytes producing precisely the same kind of melanin. In the dark races, the melanocytes merely make more melanin than they do in the light-skinned people. Melanin gives Negroes great protection against tropical sunlight.

The Harvard group have called the particles—color factories—they have isolated from pigment-making cells, *melanosomes*. Melanosomes exist in the cytoplasmic lake surrounding the cell nucleus.

The scientists found that a principal ingredient in the melanosome is a protein—an enzyme called tyrosinase which converts raw materials into the pigment melanin. As the melanosomes age, the melanin (and protein which binds it) piles up in the melanosomes and the tyrosinase activity dies down. Eventually each melanosome becomes an inert mass of protein-bound melanin.

Melanin is assembled in the melanosomes pretty much as nylon and other synthetic fibers are produced—by small molecules piling up progressively in ever-larger amounts. When the melanosome becomes pure melanin-protein, the pile-up ceases.

Melanin production depends on a balance of the raw materials from which it is formed. If one raw material, phenylalanine, piles up (because of an inherited deficiency of an enzyme that converts phenylalanine to tyrosine) melanin does not form rapidly enough. In this case, the person becomes an idiot with a disease known as phenylketonuria. His coloration, always light, darkens when the balance is restored by administering tyrosine.

Melanin, the color, comes from the Greek work for black. Actually, it is light brown in color as it is produced in melanosomes. Melanin gives foxes and rabbits their protective coloration, camouflages certain moths and provides the melanin "smokescreen" ejected by squids.

(Continued on Page 274)

THE EVOLUTION OF A NEW BREED OF SWINE

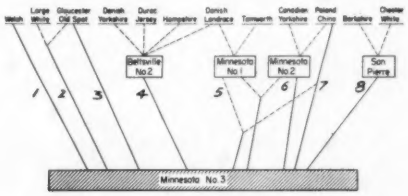
The Minnesota No. 3

LAVON J. SUMPTION, WILLIAM E. REMPEL AND LAURENCE M. WINTERS*

DURING the past 20 years animal breeding investigations have led to the establishment of a number of new breeds of livestock. Methods of breeding used in these studies have been described. Because of their likely influence upon effectiveness of selection, it is probable that the breeding methods employed are fully as important as the amount of selection practiced. The purpose of this paper is to describe the early development of the Minnesota No. 3—a new breed of swine. Because a number of new breeds of livestock have been established recently in North America, this report is unique only by virtue of some of the breeding methods employed.

Literature Review

The first report of the systematic development of an inbred line of swine from a crossbred foundation was published by Winters *et al.*[30] Although some backcrosses were made, the Minnesota No. 1 traces primarily to F_1 litters based on six individuals of the Tamworth and Landrace breeds. Performance testing of entire litters served as the basis for selection. Strict pedigree breeding was followed using no more than five boars annually during early development. The Minnesota No. 2 was developed similarly from a Canadian Yorkshire-Poland China foundation, except that backcrosses to the Poland China made a significant contribution to the line. Six animals, one Yorkshire boar and five Poland China gilts, became permanent contributors to the line[25].



ORIGIN OF MINNESOTA NO. 3
Figure 4

The eight breeds contributing to the development of Minnesota No. 3 are shown in the chart above.

The Danish Landrace has been combined with older United States breeds to form new breeds[12,19,33]. In general, the original crosses were followed by considerable backcrossing to one or several parent breeds to establish the foundation. Selection was based on the performance of tested samples from representative litters. The number of foundation litters farrowed and the number of sires used during early development was relatively small in all cases. The Palouse breed originated from crosses of the Landrace and Chester White[11]. This line traces to more foundation animals than earlier new breeds; furthermore, more sires were used to produce the early generations. Development in early segregating generations was based largely on *inter-se* mating.

The Canadian Department of Agriculture developed the Lacombe breed from a Landrace, Chester White and

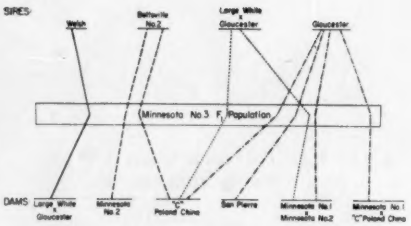
*Associate Professor of Animal Husbandry, University of Nebraska; Associate Professor of Animal Husbandry, University of Minnesota; and deceased, formerly Professor of Animal Husbandry, University of Minnesota, respectively. Paper No. 4533, Scientific Journal Series of the Minn. Agr. Exp. Sta., in cooperation with the U.S.D.A., A.R.S., A.H.R.D., Regional Swine Breeding Laboratory, Ames, Iowa. This research was supported in part by the Louis M. and Maud Hill Family Foundation, St. Paul, Minnesota.

Berkshire foundation for the specific purpose of commercial crossbreeding with the Yorkshire[6]. Use of a high sire-dam ratio in a large population has conditioned a low rate of inbreeding. Selection has been especially rigorous for carcass merit. Apparently the line has met expectations in crossbred combinations.

Essentially the same methods have been used to develop a "miniature" population of swine which has shown a response to selection for small size[7]. The generation interval has been reduced by regularly farrowing gilts at nine to 10 months of age.

Methods utilized in forming the Santa Gertrudis breed were described by Rhoad[22]. Adaptability of Brahman-Shorthorn and Brahman-Hereford crosses was compared with existing stocks before development was initiated from a Brahman-Shorthorn foundation. Selection was based primarily on performance on southwestern United States range, although feed lot testing of more than 200 bulls was undertaken in 1954 as an additional guide to sire selection. Single-sired and multiple-sired herds have contributed to breed formation. Population size has been maintained above 5,000 for 25 years.

Procedures designed to induce natural selection for range adaptability have



MINNESOTA NO. 3 F_1 POPULATION

Figure 5

The breed combinations producing the F_1 of Minnesota No. 3 are shown graphically.

TABLE I. Foundation contributors by breed, inbreeding and number of contributing F_1 progeny

Animal no.	Breed: Sires	Breed symbol*	Fx	Contributing F_1 progeny: Males	Females
1	Welsh	(W)	.07	5	1
531	Gloucester Old Spot	(G)	.01	4	8
2451	Beltsville No. 2	(B)	.38	0	3
521	Large White × Gloucester	(LG)	.00	1	4
	Dams				
B906	Minnesota No. 2	(2)	.28	0	1
B579	"C" Poland China	(C)	.26	2	1
B679	"C" Poland China	(C)	.41	1	1
B945	"C" Poland China	(C)	.25	0	1
B992	"C" Poland China	(C)	.25	0	2
C93	"C" Poland China	(C)	.27	0	1
B510x	San Pierre	(S)	.16	2	1
C324	Minnesota No. 1 × Minnesota No. 2	(12)	.00	0	2
C333	Minnesota No. 1 × Minnesota No. 2	(12)	.00	0	1
C334	Minnesota No. 1 × Minnesota No. 2	(12)	.00	0	2
C302	Minnesota No. 1 × "C" Poland China	(1C)	.00	0	2
527	Large White × Gloucester	(LG)	.00	5	1

* Symbol for breed names used in later reference.

been applied during the development of Beefmaster cattle[16]. These methods include exclusive use of multiple-sire mating and rigid culling on the basis of growth, annual cow productivity, and docility.

Russian workers have reported the performance of 300 F_1 Friesian $\times$ Tagil heifers being used in the development of a new breed of dairy cattle[2]. Korkman's observations[15] indicate the broad scope of work on breed development being conducted in the U.S.S.R., with emphasis on crossbred foundations, performance testing, and suitable methods for disseminating superior stock.

Materials and Methods

The Minnesota No. 3 was initiated to apply findings gained in the development of the Minnesota No. 1 and No. 2 and to establish a genetically diverse line for use in rotational crossbreeding. The line originated from a crossbred foundation of eight breeds (Figure 4). This approach was a deliberate attempt to create a high initial level of genetic variability to augment effective selection. It was desired to practice selection in the new population for the more favorable genes contributed by each breed. A brief description of the parent breeds is given:

Welsh—a British breed similar in type and performance to the Landrace. One imported boar was used.

Gloucester Old Spot—a minor British breed, recognized for its foraging ability and ruggedness in difficult environments. One purebred boar, an offspring of imported stock, was used.

Large White—known for its carcass merit and prolificacy, contributed through a crossbred boar and gilt that were sired by an imported Large White boar and out of a Gloucester Old Spot dam.

Beltsville No. 2—developed by the U.S.D.A., based upon approximately 58 percent Large White, 32 percent Duroc, 5 percent Danish Landrace and 5 percent Hampshire. One boar bred by the Beltsville Station contributed.

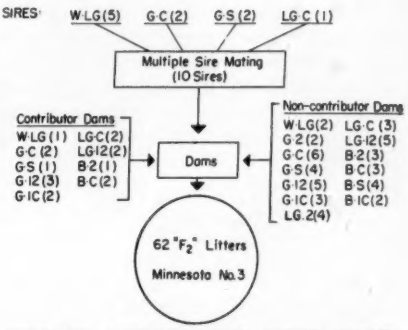
Minnesota No. 1—a new breed, approximately 52 percent Tamworth and 48 percent Landrace, characterized by high fertility, mothering ability and growth rate. The Minnesota No. 1 contributed to the female foundation stock in crossbred combinations with the Minnesota No. 2 and the "C" line Poland China.

Minnesota No. 2—an inbred line with a foundation of 40 percent Canadian Yorkshire and 60 percent inbred Poland China, initiated specifically for crossbreeding with the Minnesota No. 1, producing lean carcasses. No. 2 females contributed directly and in crossbred combinations.

"C" Line Poland China—a synthetic line composed of six original inbred

TABLE II. Estimated breed composition of generation five

Breeds	% contribution to the line
Beltsville No. 2	6.8
"C" Poland China	20.7
Gloucester Old Spot	31.4
Large White	11.8
San Pierre	5.0
Welsh	13.5
Minnesota No. 1	5.7
Minnesota No. 2	5.1



SECOND GENERATION GENE POOL

Figure 6

The number of different breed combinations that contributed to the gene pool of the F_2 are shown in the chart.

lines developed at the Minnesota Station. Although generally low in fertility and survival, when crossed with the Minnesota No. 1 this line produced lean carcasses approaching those of the Minnesota No. 2 crosses in merit. Inbred sows and Minnesota No. 1 × "C" crossbred gilts were used.

San Pierre—a line started on a private Indiana farm from a Berkshire-Chester White foundation. High fertility and heavy birth weight are particularly useful traits of this line. Inbred San Pierre females were used as F_1 parents.

Four boars sired the 37 F_1 litters in 1952, as indicated above (i.e., Beltsville No. 2, Gloucester Old Spot, Large White × Gloucester Old Spot and Welsh). Each boar was allotted a representative group of gilts. The female stocks used were those intended as the primary foundation of the Minnesota No. 3 ("C" Poland China, San Pierre and Large White × Gloucester) and additional inbreds and crosses (Minnesota No. 1, Minnesota No. 2, Minnesota No. 1 × Minnesota No. 2, and Minnesota No. 1 × "C" Poland China). Because of the primary purpose in establishing the line, the four foundation boars were subsequently test-crossed on Minnesota No. 1 and Minnesota No. 2 females with satisfactory results in all cases. Therefore, progeny of all four sires were allowed to contribute to the line. Nine of a total of 18 F_1 breed combinations ultimately contributed to the foundation (Figure 5).

MINNESOTA NO. 3 EAR TYPES
Figure 7

Some of the characteristics of the Minnesota No. 3 breed are illustrated. *A*—Large, coarse ear type, protruding forward; *B*—large, coarse ear type, semi-erect, widely set; *C*—fifth generation boars.

TABLE III. Generation intervals, years

Generations	1–2	2–3	3–4	4–5
	.95	.98	.98	1.01

TABLE IV. Farrowing record of fifth generation gilts bred in test lots

Gilt no.	140-day wt., lbs.	Approximate age at breeding, days	Age at farrowing, days	No. born
L 935	175	139	253	5
L 936	180	143	257	9
L 937	178	136	250	7

Progeny of the English breeds (Welsh, Gloucester and Large White) were favored in the selection of F_2 parents in attempting to increase the genetic diversity between the Minnesota No. 3 and the No. 1 and No. 2. Five of the 10 selected F_1 boars were Welsh $\times$ (Large White $\times$ Gloucester) crosses. The remainder of the selected boars were sired by either one-half Gloucester or Large White $\times$ Gloucester males. Conversely, the contribution of the Minnesota No. 1 and Minnesota No. 2 through the selected females was deliberately restricted.

A distinct departure from previous procedure was instituted at this point. Multiple-sire mating was used from the F_2 through the F_5. Sixty-two F_1 gilts were bred for F_2 litters. Ten F_1 boars were exposed simultaneously and continuously to the 62 gilts for 21 days. A detailed consideration of the relative merits of multiple-sire mating in swine has been presented[27].

The number of different breed combinations that contributed to the gene pool of the second generation (breed abbreviations as given in Table I) are indicated in Figure 6. No additional outside stock was introduced after the foundation was initiated. All matings have been made between animals of the same generation. No backcrossing was practiced at any time.

A fundamental objective of this project was to practice selection within a larger population than was used to develop previous lines at Minnesota. The numbers of sires and dams were increased materially. Greater population size was deemed useful in (1) increasing the probability of producing favorable gene combinations in sufficient numbers to have a significant effect upon selection progress, and (2) retarding the advance of inbreeding. Population size was maintained above 50 litters from the F_2 through the F_5.

The system of management and testing was designed for efficiency in handling a large population. Multiple-sire mating was used in an attempt to place natural selection pressure on mating efficiency among boars. Gilts of the second, third and fourth generations were bred to farrow at less than 12 months of age in an attempt to place selection pressure on early sexual maturity, and gilts that failed to conceive during one estrus cycle were culled. The line was maintained on a gilt-litter basis so that (1) the generation interval could be minimized, and (2) age-of-dam effects would not influence differences in litter performance. Spring litters were farrowed in individual houses on test pastures with one litter per pasture lot. A minimum of special post-natal care was provided in an attempt to practice natural selection for mothering ability among gilts.

With the exception of the second generation, the entire population has been performance tested. Individual body weights were taken at 56 and 140 days, and rates of gain were calculated from the growth between weighings. Feed consumption data were obtained for each litter as a group. Carcass data were collected on samples of non-selected gilts fed out in large groups after litter testing. Estimates of carcass lean and fat percentages were obtained by the method of Lu *et al.*[17] Inbreeding and relationship coefficients were estimated using a modification of Wright's formula.

In general, selection was based on performance records applying independent culling levels for fertility, survival, growth rate and feed efficiency. A limited amount of visual selection was applied for apparent carcass merit, but backfat probe data were not taken until after this study was completed.

Results

Foundation contributors to the Minnesota No. 3 are listed in Table I, giving the number of F_1 progeny that each parent contributed to the line. Sixteen individuals from a possible total of 41 parents became permanent contributors. The proportionally high contribution of the English breeds is evident from reference to Table II. It was estimated that

TABLE V. Performance of the Minnesota No. 3 by years (means and phenotypic standard deviations)

	Generations									
	1		2		3		4		5	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
No. of sires used	4		10		11		12		10	
Litters farrowed	37		62		52		55		61	
Litters tested	33		29		52		52		56	
Age of dam, days	413		350		358		362		366	
Fx dam	0.21		0.00		0.07		0.10		0.12	
Fx litter	0.00		0.07		0.10		0.12		0.14	
No. born alive	7.9	2.2	9.7	2.1	8.8	2.2	8.6	2.9	8.5	2.6
No. weaned	6.0	2.1	7.9	2.3	7.7	2.2	7.6	2.7	7.0	2.4
56-day wt., lbs.	34.0	5.3	36.9	5.8	41.0	5.6	43.3	5.2	45.2	5.6
140-day wt., lbs.	146.7	21.3	179.7	18.8	171.6	17.3	173.1	16.5	181.8	17.0
Rate of gain, lbs.	1.34	.21	1.66	.19	1.55	.17	1.55	.16	1.62	.16
Feed/cwt. gain	*		306		340		315		322	

* No data collected.

56.7 percent of the genes in the fifth generation population were derived from the Welsh, Large White and Gloucester breeds, collectively, and less than 11 percent came from the Minnesota No. 1 and No. 2.

Wide variation in superficial traits (e.g., hair and skin color; head and body type; size, shape, thickness and set of ears) have been observed during the early generations. Some segregates in each generation have closely resembled one or another of the contributing breeds. A majority of the pigs showed resemblance in head and body type to several parent breeds. In addition, new types have appeared that were not similar to any of the foundation parents. Most of the F_2 pigs were white because of the disproportionate use of boars of the British breeds, but there has been a consistent increase in the relative number of colored pigs, primarily because only boars with some degree of hair and skin pigment were selected after the third generation. None of the original stocks exhibited the belting factor; however, this has appeared with variable frequency since the F_3. Longitudinal striping appeared in the hair coat of one contributing F_3 gilt, and variable degrees of striping have appeared in each subsequent generation. Figure 7 provides limited evidence of the variations in color and type present in the fifth generation.

The generation interval was maintained at approximately one year (Table III). Breeding slightly early and during only one estrus has not been evaluated for possible effect on reducing age at puberty. However, each year a majority of the gilts were cycling regularly when initial selections were made at 160 to 180 days of age. Occasional littermate matings were observed in the test lots before 140 days during each generation after the F_1. One selected F_3 gilt farrowed a litter of six pigs at approximately eight months, and three fifth-generation gilts from one litter, bred by littermate boars before completion of the performance testing period, farrowed early litters (Table IV).

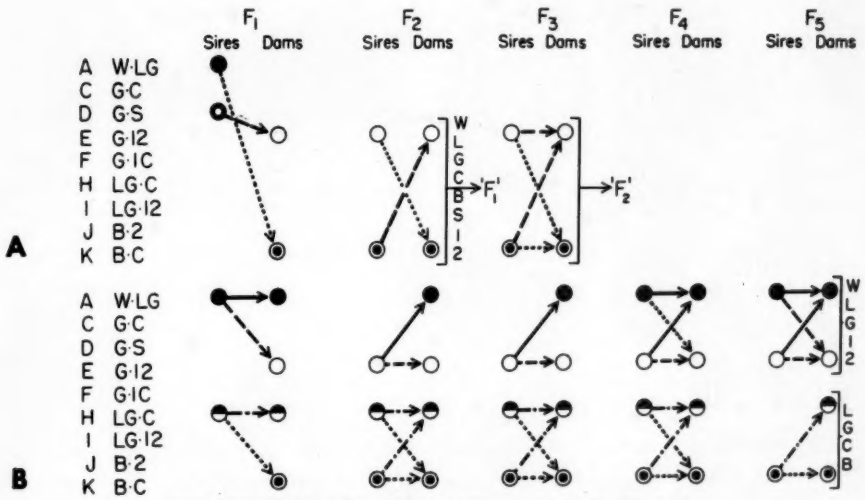


Figure 8

A—Example of recombination involving all foundation breeds; *B*—example of recombination not involving all foundation breeds.

Under the conditions of management and testing, the average performance can be considered satisfactory (Table V). Despite minimum individual attention, pig mortality has not been excessive. Weights at 56 and 140 days were consistently high, with the exception of the F_1 which was undoubtedly influenced by the fact that 29 of the 37 dams were inbred. After the F_1, yearly variation in average performance was relatively low, and there was no indication of any change in phenotypic variability for the performance traits studied. The estimated inbreeding increased to 0.14, higher than might be expected with the number of sires used per generation. This initial change can be partially explained upon reference to Table I, because of the relationship among inbred and crossbred foundation animals (e.g., the relationship among "C" Poland China sows, the Minnesota No. 1 × "C" Poland China gilt and Minnesota No. 1 × Minnesota No. 2 gilts).

The available carcass data (Table VI) are not critical because, due to variations in methods of handling the pigs measured, there were sizable differences in age and weight at slaughter. Nevertheless, the measurements indicate that the Minnesota No. 3 produces long, reasonably lean carcasses.

Discussion

Most of the breeds in widespread commercial use are the products of nineteenth century development. Methods employed that were of significant value in determining the ultimate genetic merit of these breeds are largely unknown. Herd books do indicate that some inbreeding was practiced. Korkman[15] pointed out that since the nineteenth century "the remodellers have taken a long rest." Nichols[20] recognized the need for new breeds as economic requirements change, that "a breed is successful only insofar as it . . . fills a particular ecological niche" efficiently. Use of hybridization and selection in the development of economically superior livestock for American conditions was proposed by W. M. Hays[14] of the Minnesota Experiment Station in 1906. The value of crossbred foundations has been discussed[9,21] and emphasized, especially by Winters[29].

The Minnesota No. 3 was founded on a broader genetic base than other breeds of swine developed recently[11,12,20,23]. The rationale for this approach was that if it is possible to effect more rapid genetic improvement through the development of new lines from the crossbred foundation of two complementary, genetically diverse breeds, as contrasted with continued selection within existing breeds, it is logical to assume that the same

concept can be extended. Through the combination of eight different breeds, a highly heterozygous gene pool was established. Five of the eight breeds were the object of rather intense inbreeding (Table I) and selection in immediately previous work. These stocks were considered to be useful foundation material for initiating a new phase of improvement based on the "cyclic" approach proposed by Wright[23], supported by the results of Falconer and King[10] and Robertson[20] and advanced also by Comstock[5].

The heterogeneous foundation of this population might be expected to give rise to greater phenotypic variability than less heterozygous populations. This was definitely true for superficial characters (color and morphological traits); however, comparisons of phenotypic variances for the economic traits of the No. 3 with those of the other lines in the Minnesota project did not reveal greater variation. This observation may lend support to the proposals of Anderson[1] and Comstock[5] and the data of Dobzhansky *et al.*[8] that a variety of genotypes may produce a similar array of phenotypes, masking recognition of genetic variability. Recent studies of genetic variances of the Minnesota No. 3 reveal heritability values for growth rate similar to other populations that have been studied[21] but somewhat higher values for litter size[2]. The range of segregation in color and body type during the first five generations shows no appreciable change (Figure 7). Except for the limited emphasis on selection for greater pigmentation in boars, any reduction in variability of color and form has been incidental to performance selection.

It is unlikely that there will be an early establishment of gene frequency equilibria in the Minnesota No. 3 if the potential for recombination evidenced in Figure 6 is examined. Using known matings in the F_1 and subsequent female pedigree information, it is possible to show theoretically the number of generations required before a single individual could be produced with a genetic contribution from each parent breed (Figure 8*A*). Less than three percent of the possible matings for the F_4 generation could have produced individuals possessing genes of all eight parent stocks. The relative proportion of genes from each foundation breed could vary widely. By tracing from the original background of the selected F_1 sires and dams, it can be shown that certain isolates within the population may not share genes of all the parent breeds after five generations, as illustrated in Figure 8*B*; the two isolates have Large White and Gloucester in common, but do not share Welsh, Poland China, Minnesota No. 1, Minnesota No. 2 or Beltsville No. 2. The relationship between the two groups in Figure 8*B* would be well below the estimated average of the herd.

Reference to individuals of any generation in this population by the usual terminology (F_1, F_2 . . . F_n) is arbitrary. Any zygote in the early generations may represent a true F_2 with respect to the segregation of genes for two or more breeds but could be an F_1 in relation to genes of other breeds when introduced into a specific segment of the population for the first time. Obviously a wide range of segregates could be expected to occur well after the second generation, in view of the complex genetic background involved (Figure 6).

Use of a relatively high sire-dam ratio (e.g., 1:5 to 1:6) has tended to retard the advance of inbreeding. The general approach has placed emphasis for population improvement upon selection for the expression of favorable combinations of genes from the various parent breeds. For this reason, a high level of variability should be retained in advanced generations, an essential condition for major reorganization of genotypes in view of the restriction to free recombination imposed by linkage[1,18]. It is also considered significant that no backcrosses were made in developing the Minnesota No. 3. Backcrossing is of limited value when extensive genetic recombination is desired[1].

Insofar as effective selection may be influenced by population size, it should be pointed out that the Minnesota No. 3 has been developed with a larger foundation than most previous new breeds. The line cannot be considered large by conventional plant breeding standards, yet it should be recognized that the entire herd of the No. 3 is one Mendelian

TABLE VI. Carcass data, by years

	Generations			
	2	3	4	5
No. of carcasses	18	96	49	99
Age at slaughter	148	183	190	170
Live wt., lbs.	195.5	198.7	217.7	207.0
Cold wt., lbs.	141.9	137.4	151.8	142.9
Length, inches	29.3	29.8	30.5	29.9
Average backfat thickness, inches	1.54	1.31	1.41	1.41
Estimated carcass fat, %	*	41.8	43.1	43.3
Estimated carcass lean, %	*	42.7	41.7	41.5

* No data.

population, which frequently is not the case in plant breeding (e.g., self-fertilization after initial hybridization). Several studies indicate that the probability of generating favorable gene combinations, the separate elements of which might be possessed by quite unrelated parents, is increased in larger populations at low rates of inbreeding[15,23,26,28]. When inbreeding is retarded at a given level of selection intensity, it is reasonable to assume that selection may be effective for more generations with a greater ultimate response prior to practical fixation.

The attempt was made to maximize annual progress under mass selection in the Minnesota No. 3, within the limits of the number of traits under selection and the accuracy of the selection criteria applied, by minimizing the generation interval. The average generation interval of 0.98 year for the Minnesota No. 3 is contrasted with 1.56, 1.68, and 1.43 years for the Minnesota No. 1, Minnesota No. 2, and Maryland No. 1, respectively[4,12,25].

The consistently high performance of the line during the latter four generations should be examined. One possible explanation may be found in the system of management followed. Each litter was farrowed and raised in an individual pasture lot. Litters were relatively isolated from one another during early post-natal life when most susceptible to common diseases. Competition among large groups of pigs varying greatly in age and weight is eliminated during the growing period. Furthermore, deviations in the performance of one litter or individual had a small influence on the average performance of the line because of the large population under study. The high level of heterozygosity that exists in this population may have conferred a greater range of adaptability which has tended to mask the effects of small environmental fluctuations, similar to the condition described by Robertson and Reeve[24]. It is probable that considerable heterosis is still being expressed in this population, but heterosis should not be regarded as a complicating factor under this approach to genetic improvement. The breeder would hope to capitalize on hybrid vigor in the early generations of development to obtain a high initial level of performance and a certain amount of buffering capacity against environmental variations. The attempt can be made to maintain performance comparable to the initial level through a period of slow inbreeding and intense selection. The extent to which the decline in advanced generation performance of a hybrid population of swine can be offset by selection remains to be determined.

Long-term breeding work with the Minnesota No. 3 is being continued at the University of Minnesota, and several commercial herds have been established.

Summary

Methods of breeding utilized in the early development of a new breed of swine are described. Five years' data involving 267 spring-farrowed litters and 2,345 pigs were studied.

This line is the result of combining eight breeds of diverse genetic origin. The Minnesota No. 3 is now founded on the following estimated contributions: 31 percent Gloucester Old Spot, 20 percent "C" line inbred Poland China, 14 percent Welsh, 12 percent Large White, 7 percent Beltsville No. 2, 6 percent Minnesota No. 1, 5 percent Minnesota No. 2 and 5 percent San Pierre. Sixteen foundation animals have contributed to line formation. Estimated inbreeding of the population reached 0.14 in the fifth generation.

Breeding methods employed and discussed include: (1) a broad genetic base, (2) a large population, (3) multiple-sire mating, (4) isolation rearing and natural selection for maternal traits, (5) mass selection for economic performance only, (6) minimum inbreeding, and (7) minimum generation intervals.

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✦

Pigmentation—Physical and Chemical

(Continued from Page 264)

Blue or slate-colored skin discolorations also are produced by the light brown melanin particles, the Harvard group has concluded. In this case the turbidity—a sort of muddiness—of the skin scatters the light. The same scattering phenomenon makes the sky blue at noon and red at sunset.

Among the diseases involving abnormal color are *hereditary* albinism, vitiligo and phenylketonuria; *nutritional* kwashiorkor, *hormonal* Addison's disease; forms of chemical burns and poisoning; certain infections like leprosy and such miscellaneous conditions as baldness.

Diseases involving excessive pigment are several kinds of skin spots and splotches which are inherited; porphyria, characterized by extreme sensitivity to sunlight, nutritional conditions like pellagra and sprue; several types of excessive hormone production; chemical intoxications; drug eruptions and various others.

One of the most intriguing conditions is melanoma—or cancer of the melanocytes. Since only melanocytes contain tyrosinase-bearing melanosomes, there is a possibility that a "guided missile" could be synthesized which would localize only in the melanocytes and destroy them. There have been several problems that impede the proof of this simple and reasonable hypothesis. Nevertheless, recent advances in the biology of melanocytes increase the likelihood that both diagnostic and drug methods for the management of malignant melanoma may be forthcoming.

FATTY, A NEW MUTATION IN THE RAT

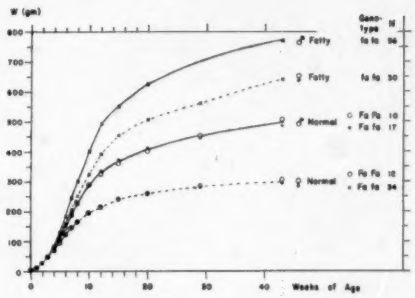
LOIS M. ZUCKER AND THEODORE F. ZUCKER*

THE mutation designated "fatty" appeared spontaneously at this laboratory in the 13M rat stock[10]. Two obese young were found in one litter and regularly, with approximately 25 percent incidence, in three more litters from the same parents. The condition appears to be due to a single recessive gene. The symbol *fa* has been chosen for the fat allele and *Fa* for the normal.

The obese condition is first noted by weight and appearance as early as three weeks of age and is very apparent by five weeks. Figure 10 shows the obvious difference in shape between adult fatties and normals. Figure 9 not only depicts the difference in the course of growth but also indicates that in body growth the heterozygotes do not differ from the normal homozygotes. Food intake observations show that fatties overeat, like other obese animals.

While the male fatties have sex organs of generally normal appearance and have occasionally been fertile, the females show a small, underdeveloped uterus and are uniformly sterile. Therefore, phenotypically normal heterozygotes of both sexes must usually be depended upon for continuing the stock.

Second to the obese appearance, the most striking sign of fatty is lactescence (milky appearance) of the blood serum. This sets in shortly after weaning, is universally found, and soon becomes very intense. Total fatty acids of the serum reach ten times the level in non-obese rats, and cholesterol and phosphatides are raised by about a factor of four. Blood lipid levels remain very high even after an 18-hour fast. On the other hand, blood sugar does not rise above the normal range in fed or fasted fatties.



MEAN WEIGHT CURVES

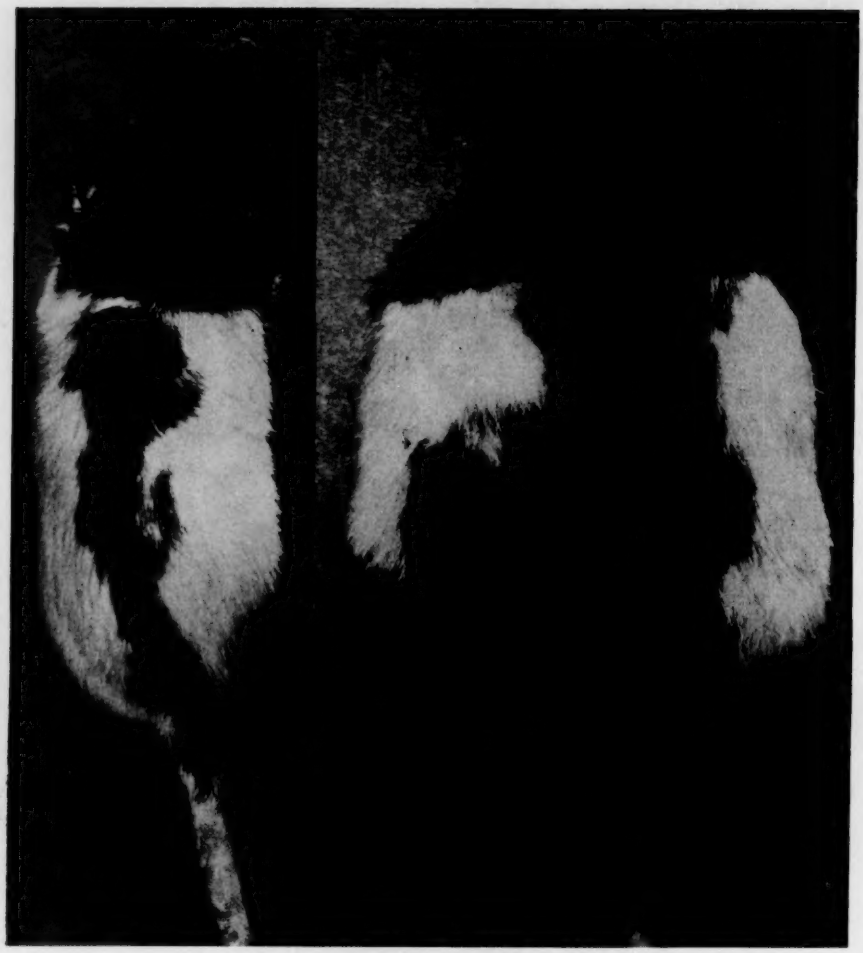
Figure 9

Mean weight curves of fatties and normal controls are illustrated graphically. The heterozygous and normal homozygous animals grow similarly.

As fatties approach a year of age, a serious kidney lesion develops, like that described by Brobeck[4] in the obese rat produced by surgical injury to the hypothalamus.

Table I records the results of various breeding experiments, all supporting the hypothesis that the fatty condition is produced by a single recessive gene. The expected yield of fatties was found for two mating types: one-half of the animals produced in the mating type of line 1 and one-fourth of those produced in the mating type of line 3 were fatties. The genetic constitution of the phenotypically normal offspring produced by each mating type was explored: for lines 1 and 2 the offspring should be, and were, all heterozygotes; for line 3, two-thirds of them should be heterozygotes and of the 24 tested, 14 proved to be so; for line 4, one-half should be heterozygotes and of the 27 tested, 14 proved to be so.

*Laboratory of Comparative Pathology, Stow, Massachusetts. This investigation was supported by research grants C-4241 and A-4085 from the National Institutes of Health.



BROTHER AND FATTY

Figure 10

A normal rat is shown with his fatty brother. They are 10 months of age and weigh 447 and 1035 grams, respectively.

Various other fat laboratory animals are known. There are several strains of obese mice, including the hyperglycemic (*obob*)[7], the adipose (*adad*)[5], and heterozygotes of yellow ($A^{y}a$)[6]. These three genes are all different. There are also obese mice of the CSH silver strain[9], and an obese New Zealand strain[2], which differ physiologically or metabolically from the obese hyperglycemic.

Rats can become obese when certain hypothalamic lesions are surgically produced[4]. In such rats Brobeck has found a marked hyperphagia but only moderate rises in serum fatty acids and cholesterol, which do not exceed a factor of two. Thus it appears that this experimental obesity is due to inactivation of the hypothalamic "satiety center," and, in contrast to the fatties, there is no outstanding abnormality in lipid metabolism. A corresponding condition can be caused in mice either by surgically produced hypothalamic lesions or by the injection of goldthioglucose[3]. Here, also, there is no marked hyperlipemia, and food restriction prevents excessive fat accumulation. Extreme obesity can be produced in rats by feeding certain high-fat diets[8]. This is called nutritional obesity and again the deciding factor is simply excessive food intake. The rat fatties, and the hyperglycemic obese mice[1], have a more basic metabolic disturbance than simply an increased appetite. When restricted to a normal food intake, they are still obviously fat in appearance. Therefore, in these two cases, it is indicated that there is an abnormality in intermediary fat metabolism.

As a subject of investigation, obesity is currently of interest in a number of respects. Every occurrence in experimental animals of a condition of interest in human medicine offers new useful channels of investigation. It may be noted in this connection that in spite of a blood cholesterol level four times that ordinarily encountered in rats, the aortas at a year of age have so far shown no signs of atheromatous plaques.

Summary

An obese rat mutation, *fa* (called fatty), is described. Breeding experiments show that it is due to a single recessive gene. The obesity is apparently of metabolic origin, being associated with obviously deranged lipid metabolism leading to very high blood lipid levels.

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TABLE I. Breeding experiments*

Matings	Offspring				Breeding tests of phenotypically normal offspring		
	Litters	Rats	Fatties (*fafa*) Obs.	Est.	Tested	Heterozygotes (*Fafa*) Obs.	Est.
$\frac{fafa}{3} \times \frac{Fafa}{5}$	6	36	19	(18)	8	8	(8)
$\frac{fafa}{1} \times \frac{FaFa}{2}$	2	16	0	(0)	12	12	(12)
$\frac{Fafa}{54} \times \frac{Fafa}{54}$	49	381	94	(95)	24	14	(16)
$\frac{Fafa}{17} \times \frac{FaFa}{25}$	26	182	0	(0)	27	14	(13.5)

* Table entries represent numbers of rats or litters; estimated numbers are in parentheses. Genotype assignment: *fafa*, fatty; *Fafa*, phenotypically normal capable of producing fatty young; *FaFa*, phenotypically normal incapable of producing fatty young.

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✦

Tooth Decay Inherited?

Some persons may be born with inherited resistance to tooth decay, a Michigan State University research team reported recently to the International Association for Dental Research.

Dr. Sam Rosen, now at Ohio State University, reported on a series of experiments conducted to determine the role of heredity in the development of dental caries. Other participants were Dr. Gerald T. Coleman, Dr. Anand C. Sawant, Dr. Harrison R. Hunt and Dr. Carl A. Hoppert.

The researchers used two strains of rats, one inbred to be susceptible to caries, and one resistant to development of caries. They cross-bred the strains and studied the offspring.

Usually, Dr. Rosen said, the offspring resembled the caries-resistant parent slightly more than the caries-susceptible parent, indicating a tendency for the gene controlling resistance to be dominant.

There was no difference, they found, whether the mother was caries-resistant or caries-susceptible. This suggests that the progeny's hereditary constitution, determined at conception, was responsible for the degree of caries susceptibility, and that both parents contribute to it.

✦

Correction—Inheritance of Taillessness in Manx Cats

An error appeared in the recent paper by Neil B. Todd, "The Inheritance of Taillessness in Manx Cats" (*Jour. Hered.* 52:228-232. 1961). On page 229, the first sentence in the fourth paragraph of column one should read "Data on the reciprocal cross of a tailed female to a Manx male are scant" rather than ". . . of a tailed male to a Manx female . . ."

—The editors regret the error.

LINKAGE RELATIONSHIP OF THE WADDLER GENE IN MICE

With Evidence for Temperature Effect on Crossing-Over

CHAI H. YOON*

THE expression and genetics of the new neurological mutation named waddler (gene symbol *wd*) have been reported previously[8]. During the course of inbreeding the waddler strain (strain symbol Wd) for other experimental purposes, it was found that gene *wd* is linked with brown (*b*) in linkage group VIII, and that high temperatures have a marked effect on the crossing-over between *wd* and *b*. The breeding data and their interpretation are presented below.

Materials and Methods

Linkage between wd and b

The original inbreeding started by crossing homozygous brown normal mice with homozygous agouti waddler mice, and the F_2 generation was produced from their F_1. If it is assumed that *wd* is linked with *b*, then the mating type that produced the F_2 may be symbolized as $+\ b/wd + \times +\ b/wd\ +$ (repulsion). From six such F_1 matings, 158 F_2 mice were obtained. As shown in Table I, there was a deficiency of the double recessive type in comparison with the 9:3:3:1 ratio expected on the basis of independent assortment. Although the chi-square value for joint segregation that indicates linkage[4] was statistically not significant and the figures were not so accurate as might be desired (many mice were lost before classification since the test was not set up for linkage), evidence for the linkage between *wd* and *b* was strong. Therefore, an experiment was started with crosses between homozygous agouti normal and homozygous brown waddler mice. The F_1 mice from these crosses were then mated to each other to produce the F_2 generation. Animals were first classified at the age of two weeks and several additional times before weaning. The mating type in this experiment that produced the F_2 may be symbolized as $++/wd\ b \times ++/wd\ b$ (coupling). From 12 such F_1 matings, 256 F_2 mice were obtained. As shown in Table I, the chi-square value for joint segregation was statistically significant at the 0.1 percent level. It was, therefore, concluded that *wd* is linked with *b* in linkage group VIII. The recombination values in Table I were computed for the first and the second F_2 data, using the maximum likelihood method described by Mather[4].

The latest linkage map compiled by Green and Dickie[2] indicates six marked loci in linkage group VIII, all within a distance of about 14 cross-over units. In order to determine the exact location of *wd*, Pintail (*Pt*) was obtained from the Roscoe B. Jackson Memorial Laboratory and three-point crosses were made as follows: $+\ b\ wd/+\ b\ wd \times Pt\ ++/+++$. The F_1 females with *Pt* were crossed to $+\ b\ wd/+\ b\ wd$ males to produce the backcross generation. From 14 such matings 163 mice were obtained. The distribution of phenotypes is shown in Table I. The chi-square value for joint segregation for *wd* and *b* was 19.94 which again is statistically significant at the 0.1 percent level, indicating linkage. The recombination values between these three genes, *Pt*, *b*, and *wd*, were very close to the values obtained in the F_2 data

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for *wd* and *b*, and as reported by Hollander and Strong[3] for *Pt* and *b*. These values indicate the order of the genes to be *Pt, b, wd*.

Since the recombination value between *Pt* and *b* is rather small, the following facts were considered in the determination of the order of the three genes: Judging from the data presented thus far, *wd* should be either to the right of *b* or to the left of *Pt*. Assuming that the order of the three genes was *wd, Pt, b*, then only double cross-overs would result in *Pt b wd* or +++, and only single cross-overs between *Pt* and *b* would produce *Pt b* + or ++ *wd*. However, none of the single cross-over types were recovered. On the other hand, if the order of the genes was *Pt, b, wd*, then single cross-overs between *Pt* and *b* would produce +++ or *Pt b wd*, and only double cross-overs would result in *Pt b* + or ++ *wd*. Since +++ and *Pt b wd* types alone were recovered and since it is highly unlikely that there were six double cross-overs while there were no single cross-overs, it was concluded that the determination of the order of genes as above is correct. Taking the weighted mean, 30.9 ± 2.5 percent, of the recombination values in the second F_2 and the backcross data, a simple linkage map was constructed as shown below. The first F_2 data were not used for the computation because of their inaccuracy and the computation of the weighted mean

TABLE I. Results of linkage tests with brown *(b)* and Pintail *(Pt)*

Types of matings	F_2 segregation				Total	Recombination value between		Analysis of χ^2	
$\frac{+\ b}{wd\ +} \times \frac{+\ b}{wd\ +}$	$\frac{+\ +}{+\ +}$	$\frac{+\ b}{+\ +}$	$\frac{wd\ +}{+\ +}$	$\frac{wd\ b}{+\ +}$				Segregation for +, *b*	0.01
								Segregation for +, *wd*	3.72
								Joint segregation	2.05
Expected 9:3:3:1	88.9	29.6	29.6	9.9				Total	5.78
$\frac{+\ +}{wd\ b} \times \frac{+\ +}{wd\ b}$	155	33	33	35	256	*wd* & *b*	29.4±3.5	Segregation for +, *b*	0.33
								Segregation for +, *wd*	0.33
								Joint segregation	32.12*
Expected 9:3:3:1	144	48	48	16				Total	32.78*

Types of matings	Backcross segregation				Total	Recombination value between	
$\frac{+\ b\ wd}{+\ b\ wd} \times \frac{Pt\ +\ +}{+\ b\ wd}$	$\frac{Pt\ +\ +}{+\ b\ wd}$	$\frac{Pt\ +\ wd}{+\ b\ wd}$	$\frac{Pt\ b\ wd}{+\ b\ wd}$	$\frac{Pt\ b\ +}{+\ b\ wd}$			
	60	32	3	0			
	$\frac{+\ b\ wd}{+\ b\ wd}$	$\frac{+\ +\ +}{+\ b\ wd}$	$\frac{+\ b\ +}{+\ b\ wd}$	$\frac{+\ +\ wd}{+\ b\ wd}$			
	44	3	21	0			
					163	*Pt* & *b*	3.7±1.5
						Pt & *wd*	36.2±3.8
						wd & *b*	32.5±3.7
					For *wd* and *b*, χ^2 for joint segregation		19.94*

*Statistically significant at the 0.1 percent level.

TABLE II. Offspring produced by + *b wd*/+ *b wd* × *Pt* + +/+ *b wd* matings designed to establish an inbred strain with *Pt* and *b* in heterozygous condition, bred at higher temperature

Pt	+ +	+ *wd*	*b* +	*b wd*	Total	Recombination value between *wd* and *b*	χ^2
With *Pt*/*pt*	25	22	—	1	48		
With *pt*/*pt*	2	2	21	23	48		
Total	27	24	21	24	96	46.9±5.1*	
Expected 1:1:1:1	24	24	24	24	96		0.75†

*The difference between this and the expected recombination value, 30.9 percent, is statistically significant at the 1 percent level.

†This is statistically not significant at the 5 percent level.

was made according to the method described by Finney[1].

30.9

Pt *b* *wd*

Effect of high temperatures on crossing-over

When the linkage experiments were over, animals were chosen at random from the backcross generation, and six $+$ *b wd*/$+$ *b wd* male $\times$ *Pt* $++$/$+$ *b wd* female matings were made in order to obtain an inbred strain with *Pt* and *wd* in heterozygous condition. These matings were allowed to reproduce in a room where the temperature was, on the average, about 10° C. higher than the normal room temperature. However, no accurate recording of the temperature of the room was made. When 93 mice were produced in 13 litters during a period of about three months and the animals were classified, rather unexpected results were obtained as shown in Table II. No evidence for the linkage between *wd* and *b* could be found and these two genes apparently behaved as though they were independent. In addition, two out of five recombinants involving crossing-over between *Pt* and *b* were double cross-over types which were absent in the three-point crosses.

In order to explain these aberrant results, four relatively simple assumptions were made as follows: (1) there may not exist any linkage between *wd* and *b*; (2) the aberrant results may be due to a sampling error; (3) there may have been a mutation affecting the crossing-over frequency between *wd* and *b*; and (4) the higher room temperature may have a marked effect on the crossing-over frequency between *wd* and *b* in female mice. The first possibility can be safely eliminated in view of the fact that two carefully carried out experiments showed chi-square values for joint segregation significant at the 0.1 percent level. Because of a rather small sample in this inbreeding experiment the second possibility cannot be entirely ruled out but it is highly unlikely. The third possibility can be easily eliminated because mice used for the original six matings were from several different parents that produced the backcross generation in Table I. The last interpretation is in conformity with the reports by Plough[5, 6] and Stern[7]. Both of these authors found that temperatures have marked effects on crossing-over frequencies in *Drosophila melanogaster*. They reported that the higher temperature increases the crossing-over frequencies in various chromosomes of the organism. Therefore, it was concluded that the higher room temperature in which oogenesis of female parents took place increased the crossing-over frequency between *wd* and *b*.

Summary

Evidence was presented for the linkage between *wd* and *b* in linkage group VIII and the data analyzed to show the order of genes to be *Pt*, *b*, and *wd*. Evidence was also introduced to show that higher temperature increases the crossing-over frequency between *wd* and *b* in female mice.

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LINKAGE AND SEX LIMITATION OF SEVERAL LOCI IN THE HOUSEFLY

ROBERT L. SULLIVAN*

GENETIC studies of the housefly, *Musca domestica* L., have not been extensively pursued up to the present time, but interest in housefly genetics has increased in recent years with the development of insecticide resistance in these insects. Investigations on the genetics of the housefly have been performed recently in the laboratories of Milani[6] in Italy, Tsukamoto in Japan, and in this laboratory. The investigations reported here have involved the use of marker stocks obtained from other laboratories and the development of mutant stocks by inbreeding of field and laboratory strains.

Linkage studies involving a single pair of chromosomes are treated in this paper. A spontaneous translocation, in which the expression of normally autosomal characters is limited to the female fly, is described and discussed. Linkage distances of several loci have been determined as a consequence of the study of this translocation, herein designated T(Y,II).

Materials and Methods

Rearing techniques

Both single pair and mass culture breeding of houseflies have been used in this work. For single pair cultures the following technique, modified from Barbesgaard and Keiding[2], has been used. The tops of inverted, cylindrical, one-pint ice-cream cartons are removed and covered with Saran wrap, which allows a transparent window for observation. A small aluminum foil cup containing milk and cellucotton, to prevent drowning, is placed on the removable bottom. Then a pair of adult flies is introduced into the carton. The milk cups are changed daily by placing a fresh cup on another bottom while taking advantage of the flies' tendency to go to the top of the box when disturbed. On the fourth day of adult life, after mating has occurred and females are ready to lay eggs, the flies are provisioned with one cup containing milk and a second containing CSMA larval medium[1]. It has been the author's experience that in virtually all cases the female fly will lay her eggs in the cup of medium rather than in the moister milk. The cups of medium are changed daily, and any cup which contains eggs is placed in a one-half-pint milk bottle two-thirds full of CSMA medium and capped with paper towelling. Larval development then proceeds in the bottles at 25° C. Up to 150 adult flies will develop from single clutches of eggs resulting from outcrosses. Normally only the first two clutches of eggs produced are saved, since the frequency of egg laying tends to decrease rapidly. However, the number of flies obtained from the first two clutches is sufficient for most tests.

Mass cultures are reared in standard screen cages one foot in each dimension, with a glass front and muslin sleeve at the back. Eggs from mass cultures are collected and placed in quart jars which yield up to 800 flies per bottle. Adult flies in the culture bottles are anesthetized with carbon dioxide to facilitate transfer to etherizing bottles where sufficient immobilization is effected for examination.

Sources of the strains

The strains used in these experiments

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were obtained from several other laboratories. Four recessive markers were used: brown body color (*bwb*) and ocra eye color (*ocra*) obtained from Dr. R. Milani, green eye color (*ge*) and white eye color (*w*) from Miss Louise Zingrone of the Illinois Natural History Survey, Champaign. Dr. W. N. Bruce of the Illinois Natural History Survey also furnished the D^{+++} strain which was used in some of the testcrosses.

A mutation to brown body color occurred in an inbred strain which had been maintained in this laboratory for seven generations by brother-sister matings. Both male and female brown-bodied flies were found in one of the six cultures of this generation. The phenotypes in this one culture were: wild type 58 ♂♂ and 33 ♀♀, brown body color 5 ♂♂ and 1 ♀♀. Subsequent generations reared from the heterozygous brothers and sisters of these brown flies resulted in an anomalous line. A strain was established in which all male flies show the wild phenotype and all females are brown-bodied. This strain will hereafter be called T(Y,II).

The mutants brown body color, green eye color, and white eye color are borne on the same chromosome, chromosome 2 (Hiroyoshi[4]). The mutant ocra eye color is on chromosome 3. The three eye-color mutants all have pleiotropic effects. The adult testes and larval Malpighian tubules in these three mutants are virtually colorless, in contrast to brown testes and yellow Malpighian tubules found in wild-type flies.

Results and Discussion

Linkage relationships

Linkage tests were made of the *bwb*, *w*, and *ge* loci. These three genes are all located on the second chromosome. Tests of the distance between loci yielded female crossover data of 20.3 percent between *bwb* and *w*, 28.2 percent between *bwb* and *ge*, and 39.1 percent between *w* and *ge*. Tables I and II give the results of the F_2 of crosses between *bwb* and eye color loci, the testcross of the female carrying the two markers in repulsion crossed with the double recessive male, and the testcross of the male carrying the markers in repulsion to the double recessive female. Table III gives the results of tests between *ge* and *w*, including only the testcross data.

The test for male crossing-over between *bwb* and *w* yielded three crossover types and one mosaic in a total of 10,863 flies. The crossover percentage for males calculated from these data was 0.03 percent. The test for male crossing-over between *bwb* and *ge* gave one crossover type and five mosaic individuals in a population of 5,371 testcross progeny, i.e., 0.11 percent crossing-over in the male. There were no male crossover types observed in the tests between white and green. However, the *w ge* crossover type could not have been differentiated from the white phenotype had it occurred. It is to be understood that alternative possibilities, such as non-disjunction,

TABLE I. F_2 and testcross linkage data of the cross of brown body with white*

Genotypes of parents		Phenotypes of offspring				
♀♀	♂♂	+	*bwb*	*w*	*w bwb*	mosaic
+ *bwb*/*w* +	+ *bwb*/*w* +	3,439	1,502	1,673		
+ *bwb*/*w* +	*w bwb*/*w bwb*	1,063	4,146	4,104	1,033	
w bwb/*w bwb*	+ *bwb*/*w* +	3	5,363	5,496		1
Crossing-over frequency 20.3 percent						

* Data obtained in cooperation with Mr. T. Hiroyoshi.

TABLE II. F_2 and testcross linkage data of the cross brown body with green

Genotypes of parents		Phenotypes of offspring				
♀♀	♂♂	+	*bwb*	*ge*	*bwb ge*	mosaic
+ *bwb*/*ge* +	+ *bwb*/*ge* +	6,559	2,918	2,909	6	
+ *bwb*/*ge* +	*ge bwb*/*ge bwb*	829	1,937	1,961	700	
ge bwb/*ge bwb*	+ *bwb*/*ge* +		2,674	2,697	1	5
Crossing-over frequency 28.2 percent						

are not ruled out in these tests, but for ease of description the exceptional recombinations are referred to as male crossover types.

In the mosaic individual from the brown × white cross, the following structures were brown: head, one side of the thorax, three legs, and one wing. The remainder of the body exhibited wild-type pigmentation. From the brown × green cross a similar pigment distribution was found in the mosaic individuals.

No crossover types were found when the inbred F_2 generation of the cross between *bwb* and *w* was examined (Table I, line 1). In the case of *bwb* and *ge* (Table II, line 1), six *bwb ge* flies were found in a total of 12,392 flies from 63 single pair matings. The distribution of the *bwb ge* flies among these 63 matings was one in one family, two in another, and three in a third.

The female crossover tests of the *bwb* and *ge* loci were performed only on single pair cultures. The tests for crossing-over in the female between *bwb* and *w* were performed on both single pair and mass cultures. The two tests compare well; the 20 single pair cultures yielded a value of 20.2 percent crossing-over, while in the mass cultures this value was 20.3 percent. These data were obtained on 7,027 flies grown by mass cultures and 3,319 from single pair cultures. The pooled estimate of 20.3 percent should therefore be a reliable estimate of crossing-over in this *w-bwb* region.

The results on female crossing-over between *bwb* and *ge*, based on 5,427 flies from single pair cultures, show a scarcity of the double recessive class. The estimated crossing-over, 28.2 percent, is therefore probably low for the actual distance between these loci.

In order to determine the distance between the *ge* and *w* loci, the appearance of the homozygous double recessive individual had to be ascertained. From the F_2 of a cross between green- and white-eyed flies, two cultures were set up, one containing the green-eyed flies, the second the white-eyed flies. The green and white phenotypes could not be differentiated upon emergence, as the green flies require a period of 24 hours for complete pigment development which allows for separation from white. Therefore, all flies were isolated as virgins and separated 24 to 48 hours later according to eye color. All offspring of the white-eyed flies showed white eye color; among the offspring of the green-eyed flies a number of white-eyed individuals were found. These flies formed the basis of the *w ge* stock which was used in the testcrosses reported in Table III. The order of the three genes is therefore determined to be *w bwb ge*, with a total map distance indicated to be between 39 and 48 crossover units.

Sex limitation of the new mutant for brown body color

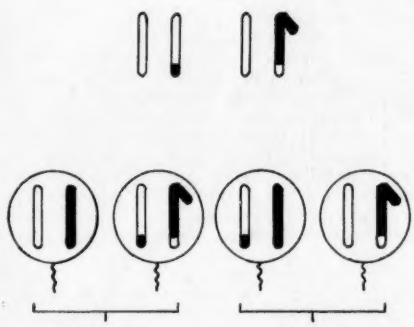
Tests to determine the mode of action of factors restricting the expression of the mutant character brown body color to the females in the T(Y,II) strain were made as follows. Data on the strain for one generation gave 3,396 wild-type males to 2,552 brown-bodied females. These data are typical of the performance of this strain, which has been maintained in the laboratory for over 20 generations in mass culture. Counts were not made in each generation, and the data given are a sample of the large number of flies of this strain which were raised during the experiment.

Female flies of the T(Y,II) strain, brown in phenotype, were crossed to males of the *bwb* mutant strain of Milani. The results of this cross yielded 100 percent brown-bodied flies in the F_1 generation, 1,446 ♂♂ and 1,333 ♀♀. The F_2 and subsequent generations of this cross continued to produce all brown-bodied flies. When males of the T(Y,II) strain were outcrossed to *bwb/bwb* females of the Italian strain, the F_1 consisted of 2,392 wild-type ♂♂ and 1,966 brown ♀♀. When female flies of the T(Y,II) strain were outcrossed to wild-type males of the D^{+++} strain, the F_1 was all wild type and the F_2 males and females gave a ratio of 2,460 wild type to 750 brown, as expected for an autosomal recessive. This same result is achieved when either male or female flies of the brown strain are tested against wild-type flies. However, when the male of the T(Y,II) strain is outcrossed to wild-type females, the F_1 and F_2 generations are all wild type. When 62 pairs of the F_2 flies were brother-sister mated in single pair cultures, the resultant F_3 cultures yielded 15 which contained wild-type males and females and brown-bodied females. The remaining 47 cultures were all wild type. The over-all ratio was wild type, 5,684 ♂♂ to 4,463 ♀♀, and 444 *bwb/bwb* ♀♀. In mass cultures of this same cross the results were: wild type, 1,116 ♂♂ to 1,029 ♀♀, and 129 *bwb/bwb* ♀♀; thus, approximately 1/16 of the F_2 flies were brown-bodied females. In a testcross of F_1 males of the above cross to *bwb/bwb* females, the offspring were all wild type, 903 ♂♂ to 957 ♀♀. When, however, the reciprocal testcross was performed, the offspring showed a ratio of one wild type to one brown in both sexes.

As an explanation of these results, a reciprocal translocation involving the Y and the second chromosome carrying the wild-type allele for the brown locus is postulated. In

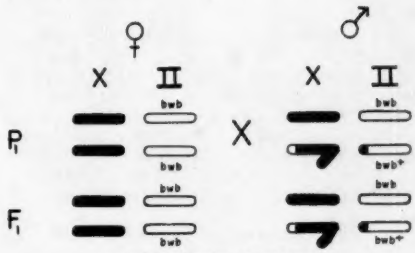
TABLE III. Testcross linkage data of the cross of green with white

Genotypes of parents		Phenotypes of offspring		
♀♀	♂♂	+	*w*	*ge*
+ *w* / *ge* +	*ge w* / *ge w*	188	473	300
ge w / *ge w*	+ *w* / *ge* +		991	1,058
Crossing-over frequency 39.1 percent				



HYPOTHETICAL TRANSLOCATION
Figure 11

The hypothetical translocation is shown above; the four types of sperm produced are shown below. The two sperm types on the left contain the entire genetic complement and would, therefore be viable. The two sperm types on the right would be lethal, one owing to the absence of a portion of the autosome and duplication of a portion of the Y, the other due to a loss of a region of the Y and duplication of a region of the autosome.



RESULTS OF CROSSES
Figure 12

The cross of a male of the T(Y,II) strain, carrying the translocation and the *bwb/bwb*$^+$ alleles, to a homozygous *bwb* female is shown in this diagram. In the resultant F_1 the male flies are of the wild-type phenotype, *bwb/bwb*$^+$, and the females are homozygous for *bwb*. Similar results are obtained with both the *w* and *ge* markers, as well as for the combinations *bwb,w* and *bwb,ge*.

Figure 11 this translocation is diagrammed. In spermatogenesis four types of sperms could be produced from this translocation, two containing the full complement of genetic material, one deficient for a portion of the Y chromosome, and one deficient for a portion of the autosome. The gamete which is deficient for a portion of the Y would also be carrying a portion of the autosome in duplicate, and the gamete which is deficient for a portion of the autosome would carry a duplication of a portion of the Y. The sperms which bore these duplications and deficiencies would, on the basis of the genetic data, produce a lethal effect. Only the sperms carrying a complete genetic complement would be viable. Dobzhansky[3] and Rhoades[6] discuss a similar translocation between the second chromosome and the Y of *Drosophila melanogaster*. The genes of the second chromosome behave as if they were in the Y chromosome, all the sons resembling the father.

The results given thus far indicate that the sons are always like the father with respect to the character *bwb*. The cross of the *bwb/bwb* female to a T(Y,II) male would be expected to give the same results as within the T(Y,II) strain, as all males would carry the wild-type allele (Figure 12). The female of the T(Y,II) strain, when outcrossed to a wild-type male, would behave as a homozygous recessive brown female, giving a 3:1 ratio in the F_2 regardless of the sex of the offspring. The cross of a wild-type female with a T(Y,II) male would yield F_1 females heterozygous for the *bwb* allele; the F_1 males would carry a *bwb*$^+$ autosome from the female parent and a wild-type allele also on the translocated chromosome. Males of this F_1 crossed to *bwb/bwb* females yielded all wild type in the testcross progeny. When the reciprocal cross of the F_1 female to the *bwb/bwb* male was performed, the testcross progeny approximated a 1:1 ratio of brown to wild type in both sexes. The F_2 results of the cross of a wild-type female to a T(Y,II) male are also as expected. Since no males in the F_1 generation are carriers of the *bwb* allele, F_2 flies are all wild type. When this cross is reared to an F_3 generation, one-fourth of the F_3 cultures contain brown-bodied females.

Outcross tests were performed to show that the brown females of the T(Y,II) strain were carrying a normal recessive allele for brown. Females of the T(Y,II) stock were crossed to males of the Lab California strain (LC) and gave these F_2 results: wild type 2,301 ♂♂, 1,070 ♀♀, and 1,058 *bwb/bwb* ♀♀. The 2:1:1 ratio indicated the possibility that the T(Y,II) female might influence the results. Subsequently the cross of the Italian brown female and the LC male was performed and gave similar F_2 results, i.e., wild type 992 ♂♂, 490 ♀♀, and 409 *bwb/bwb* ♀♀. This indicated that the LC male was responsible for the anomalous results, a conclusion which was further confirmed when the cross of the LC female to a *bwb/bwb* male was performed and the 3:1 ratio was approximated in both sexes in the F_2. Thus it appears that the LC strain also bears the postulated translocation. Three other wild-type laboratory strains which have been tested have also expressed the T(Y,II) behavior, while four have shown the normal behavior for this locus.

Other loci on the second chromosome show the effect of the translocation as well. A cross

of a homozygous white female to a T(Y,II) male gave an F_2 generation of 2,129 + ♂♂, 942 + ♀♀, and 1,024 *w/w* ♀♀. Thus it is seen that the translocation causes the absence of the phenotypes of the linked mutant loci in the males.

Male flies of the LC strain were crossed to *ge/ge* females. In the F_2 there were 2,895 + ♂♂, 1,373 + ♀♀, and 1,024 *ge/ge* ♀♀. Again the 2:1:1 ratio was approximated.

Finally strains were established in which only the females manifested both brown body color and green eye color, and likewise brown body color and white eye color. These strains have maintained themselves with all males normal and all females carrying both mutants.

Limitation of the effect of the T(Y,II) strain males to a single chromosome was checked by crosses with the *ocra* mutant. If the anomalous behavior extended to other chromosomes, then the cross of an *ocra/ocra* female to a T(Y,II) male should yield the *ocra* phenotype only in females. If, however, the effect were limited to a single chromosome, *ocra,* which is on the third chromosome (Hiroyoshi[4]), would be expected to show up in one-quarter of the F_2 flies, regardless of sex. Results of this cross gave 982 + ♂♂, 945 + ♀♀, 307 *ocra/ocra* ♂♂ and 316 *ocra/ocra* ♀♀.

Cytological studies are being conducted in order to confirm the hypothetical translocation of a portion of the Y to an autosome. Cytological reports of Stevens[9], Metz[5], and Perje[7] have shown that the housefly has six pairs of chromosomes and that the male is the heterogametic sex. The Y chromosome has been clearly distinguished in their work. In the anomalous strain reported here, six pairs of chromosomes have also been distinguished.

This strain of flies should be very well suited as a radiation tester stock. Treating males with ionizing radiations and thereafter mating them to the homozygous recessive females will allow for detection of mutations at the marked loci in the F_1 generation. Studies of this type are being carried on at the present time.

Summary

On the basis of crossover tests in which the female carried the markers in repulsion phase, the distance from the brown body color (*bwb*) locus to the white eye (*w*) locus was found to be 20.4 crossover units. The distance from *bwb* to green eye (*ge*) was determined as 28.2, and from *ge* to *w*, 39.1 units. These results give an order of *ge bwb w* for the three loci.

When males carrying the same markers in repulsion phase were tested, the amount of male crossing-over was found to be less than 0.1 percent in all cases. Several mosaic individuals were found in the testcross progenies.

The eye color mutants *ge, w,* and *ocra* were found to have pleiotropic effects on the pigmentation of the Malpighian tubules of the larvae and the testes of the adult male. In all cases the normally pigmented tissues were colorless in the mutant stocks.

Strains have been found in which any combination of the linked mutants, *bwb, ge,* and *w,* is expressed only in the female fly. A translocation between a portion of the second chromosome carrying the loci and the Y chromosome is postulated as causing this type of inheritance.

When a non-linked marker, *ocra,* is tested with T(Y,II) stock males, the F_2 generation gives a 3:1 segregation of *ocra,* as would be expected on the above hypothesis.

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A NEW JUTE HYBRID

A. S. Islam and A. Rashid*

HYBRIDIZATION between *Corchorus olitorius* and *Corchorus capsularis* has always failed to produce viable seed in the past. It was noted, however, that when *C. olitorius* was used as the female parent, some fruit set was obtained but none of the seeds germinated. Srinath and Kundu[6] could not ascribe this failure of formation of viable seed to the inability of the pollen tubes of one species to grow inside the style of the other.

After this finding, many workers tried to cross the barrier of incompatibility between the two species, but none were successful. Ganesan *et al.*[3] observed that the failure of seed-setting was due to the degeneration of the embryo and free endosperm nuclei at an early stage of development. The fact that an embryo of a few cells and a few free endosperm nuclei are formed following hybridization encouraged these authors to repeat the cross, employing techniques which were aimed at completing full development of the embryo and the endosperm.

Materials and Methods

The best varieties of both species, D-154 of *C. capsularis* and C.G. of *C. olitorius*, were obtained from the Jute Research Institute, Dacca, East Pakistan. The improved technique consisted of wrapping the stalks of the pollinated flowers with cotton wool previously soaked in various concentrations of 3-indole-acetic acid and kept wet thereafter by adding fresh solution in drops for one day. The seeds were germinated first in Petri dishes, and when they were three days old, transplanted to 6-inch pots containing moss. This practice eliminated chances of contamination by the seeds of other *Corchorus* species.

Pollen-mother-cells fixed in acetic alcohol (1:3) were studied in aceto-carmine.

Results

Reciprocal crosses were made between *C. olitorius* and *C. capsularis*, and it was observed that seeds obtained from crosses in which the hormone was applied on the stalks of the pollinated flowers appeared more well-developed than those without the treatment. The results of the crosses are shown in Table I.

The percentage of fruit set during the two successive years, as shown in Table I, was 13 and 14.7 in those crosses in which *C. olitorius* was used as the female parent. The percentage of germination was also very low. Of 365 seeds only seven germinated and of these only three grew to adult plants,

TABLE I. Results of crosses between *C. olitorius* and *C. capsularis*

Year	Parents		No. of crosses	No. of fruits harvested	Percentage of fruit set	No. of seeds	Percentage of germination	No. of adult plants	Remarks
	Female	Male							
1958	*C. olitorius* × *C. capsularis*		115	15	13.0	365	1.91	3	—
	Reciprocal		83	16	19.3	—	—	—	—
1959	*C. olitorius* × *C. capsularis*		95	14	14.7	575	—	—	Not yet grown
	Reciprocal		59	0	0	—	—	—	—

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the remaining ones having died within a few days.

In the reciprocal cross the percentage was nil in 1959. This difference was probably due to the fact that in 1958 emasculation and pollination were done on the same day, whereas in 1959 the pollination was done on the day following emasculation. Postponement of pollination by one day was adopted as a precaution since the few seeds that germinated in the cross in 1958 turned out to be selfed progeny. It may also be possible to bring about success in this combination by doing the emasculation during the small hours of the night and doing the pollination a few hours thereafter.

The hybrids have been compared with their parents in Table II.

Almost all the flowers borne in the beginning of the blossoming period fell off, but approximately 50 percent of the flowers that subsequently opened developed into fruits.

Backcross

The results of backcrosses are given in Table III.

None of the 83 backcrosses between

TABLE II. Comparison of characters of the hybrids with the parents

Character	*C. olitorius*	F_1 (*olitorius* × *capsularis*)	*C. capsularis*
1. General			
(a) Height	96.3″	36.7″; 62.5″; 76.2″	84.5″
(b) Base diameter	0.620″	0.440″, 0.435″ and 0.265″	0.720″
2. Leaves			
(a) Stipules	A pair of free lateral stipules	Stipules intermediate and fall off	Stipules small and fall off
(b) Dimensions	Large 4.5″–6.1″ × 1.3″–2.6″	Smaller and of *capsularis* type 1.7″–3.1″ × 1.5″–1.8″	Large 5.3″–6.1″ × 2.5″–3.3″
(c) B/L ratio	0.437 ± 0.011	0.539 ± 0.014; *capsularis* dominant	0.538 ± 0.006
(d) Serration	Serrated	Greater portions of the margin serrated; very little dentate; *olitorius* dominant	Dentate
3. Flower characters			
(a) Bud size and shape	Large	Intermediate; shape that of *olitorius*	Small
(b) Petals			
(i) Shape	Large, entire	Intermediate, notched in the majority of petals, *capsularis* dominant	Small, notched in center of the upper margin
(ii) Color	Yellow	Intermediate	Pale yellow
(iii) Pollen diameter	37 ± 2.4 μ	33 ± 3.2 μ; *capsularis* dominant	32 ± 2.4 μ
4. Fruits			
(a) Shape	Elongated, 2½–3″	Elongated but much shorter in length, 1½–2″; *olitorius* dominant; comparatively broader in width than the female parent	Spherical .439″ (diameter)
(b) Orientation of the ovarian wall			
(i) No. of ribs	10-ribbed	10-ribbed, *olitorius* dominant	—
(ii) Surface of the fruit	Smooth and ribs parallel	Ribs irregular with small alternate projections and depressions running along each rib; *capsularis* dominant (see Figure 13)	Irregular projections and depressions all over surface
(iii) Color of seeds	Black with greenish tinge	Brown with blackish green tinge; *capsularis* dominant	Brown

HYBRID JUTE PLANTS
Figure 13

A—One of the three F_1 hybrids (center) between *C. capsularis* (left) and *C. olitorius* (right). *B*—A branch of the hybrid with leaves showing white patches representing yellowish green areas. Note the buds which are of the *olitorius* type. *C*—A fruit of the hybrid showing irregular projections and depressions along the ribs.

capsularis and the hybrid resulted in fruit formation. The backcross involving *olitorius* as female with the hybrid yielded five fruits out of 121 crosses. No success was achieved in the backcrosses in which *olitorius* and *capsularis* were used as the male parents. The seeds in fruits resulting from backcrosses were usually empty.

Meiosis

Meiosis was studied in order to learn the extent of homology between the chromosomes of the two species and to form an idea of the contributory factors affecting the fertility of the hybrid. Detailed meiotic studies were not possible as the flower buds were saved to allow fruit formation for raising the subsequent generation. In all the diakinetic nuclei, as well as in the side view of metaphase I, seven bivalents were seen. One of the bivalents in the majority of pollen-mother-cells was observed to consist of chromosomes of unequal length. This preliminary observation indicates that sufficient homology exists between the chromosomes of *capsularis* and *olitorius* to permit the formation of seven bivalents. The fact that fruits with well-developed seeds were formed further shows that this homology is more than that existing between species with structural differences which cause the interspecific hybrids to be sterile, as in the hybrid between *Primula floribunda* and *P. verticillata*.

Discussion

Crosses between cenospecies fail in most cases because of the formation of an abscission layer in the stalk of pollinated flowers. When the

TABLE III. Results of backcrosses

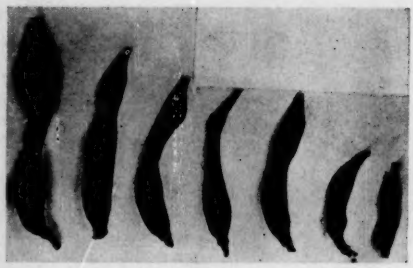
Female	Male	No. of crosses made	Fruits harvested	Percentage in terms of fruit set
1. *olitorius*	F_1	121	5	4.13
2. *capsularis*	F_1	83	0	0
3. F_1	*olitorius*	31	0	0
4. F_1	*capsularis*	11	0	0

hormone, 3-indole-acetic acid, was applied to the flower pedicel, it not only prevented the formation of an abscission layer but stimulated the full development of the embryo and endosperm in many fertilized eggs and secondary nuclei. Ganesan *et al.*[3] showed, as pointed out earlier, that the failure of seed formation in this cross was due to the degeneration of both embryo and endosperm at an early stage of development, and the hormone used in the present investigation seemed to have removed that barrier which led to the abortion of embryos, etc. The inhibitory effect which might have been caused on account of an abnormal embryo-endosperm relationship in the pollinated flower being counteracted by the hormone, the embryo and the endosperm completed their development, thus giving rise to a few fully developed seeds which germinated to produce F_1 hybrids. Dionne[2] reported recently of similar success in the interspecific crosses of *Solanum* species.

All three hybrids were weak and slow growing (Figure 13*A*)—characters which are very often exhibited by the interspecific hybrids of distantly related species. They flowered later than their parents. Both parents of the hybrids are short-day plants, and therefore the delayed flowering in the hybrids raises interesting points of discussion. Delayed blooming may be due to the slow growth of the hybrids or rearrangement of the genes responsible for photoperiodic behavior.

Another notable feature of the hybrids was the appearance on the leaves of yellowish green areas surrounded by normal green pigment (Figure 13*B*). These areas were irregular in outline and varied from leaf to leaf. The formation of such areas may be due to a disturbance in the gene arrangement responsible for the formation of chlorophyll. Such cases have been reported earlier in interspecific crosses, *e.g.*, in *Gossypium hirsutum-barbadense* hybrids[4]. Such interference in the formation of normal pigmentation was also noticed in the petals, which showed a number of irregular white streaks running from their bases almost to the apices. In Table II 13 characters have been compared. Of these the hybrids showed dominance of the female parent in four. The dominance of the male parent was exhibited in five characters, while in four characters the hybrids were intermediate.

The chromosomes of *olitorius* are somewhat different in morphology (including length) from those of *capsularis*[5]. This inequality in the length of chromosomes, however, did not prevent the formation of bivalents, and the irregularities did not seriously affect the fertility of the pollen grains, nearly 75 percent of which took brilliant stain in aceto-carmine. The eggs must have been as good as the pollen grains since the fruits contained a large number of well-developed seeds. As against 200 to 230 seeds in *olitorius* and 30 to 40 seeds in *capsularis*, the hybrids contained five to 50 normal seeds, in addition to many that were shrivelled and shrunken. Whether the F_2 progeny will show an increase in their fertility or whether they will fail to survive, as is the case with F_2 populations of many vigorous F_1 hybrids of species crosses, is difficult to say at this stage.



FRUITS OF THE SECOND GENERATION

Figure 14

Variation in the shape and size of the constricted fruits of the F_2's.

The very low percentage of fruit set in the backcrosses is contrary to expectation since 75 percent of the pollen grains of the hybrids were good, as indicated by their stainability with aceto-carmine. The very low crossing ability may be due to the same factors which cause the breakdown of hybridization between *capsularis* and *olitorius*.

Summary

By applying 300 p.p.m. 3-indole-acetic acid on the pedicels of the pollinated flowers, it has been possible to obtain three F_1 hybrids from the cross *C. olitorius* × *C. capsularis*—a combination in which no success was hitherto achieved.

These three hybrids were weaker, grew more slowly and flowered later than their parents. The hybrids showed dominance of the female parent in serration, shape of flower bud and fruit and in the presence of ribs on the fruit wall. The dominance of the male parent was noted in B/L ratio, a notch in the upper margin of the petals, pollen grain diameter, irregular markings on the ovarian wall and in the color of seeds, whereas in the character of stipules, color of petals, and size of flower bud and petals, the hybrids were intermediate.

Addendum

Since submitting this manuscript for publication, F_2 and F_3 populations have been studied. In the F_2 progenies, which consisted of 200 plants, there was much variability in respect to vigor, height, shape and size of flowers and fruits, and number and color of seeds. Particularly noteworthy was the occurrence in the majority of plants of constricted fruits, constriction usually being found in any portion between the apex and middle of a fruit (Figure 14).

The more vigorous F_2's were fairly fertile. Their seeds were grown along with those of the control female parent in plots designed for statistical analysis. The F_3 progenies consisted

of 2,850 plants of the *olitorius* type with elongate fruits and 30 plants of the *capsularis* type with more or less globular fruits; thus there was a skewed distribution of recombination types towards the female parent, as has been observed recently by Swaminathan (*Nature*, 192: 893, 1961) in the F_2 of the same hybrid combination. However, Swaminathan did not observe any individuals bearing the globular *capsularis* type of fruit. The *t* test was used to compare the performance of the selected non-branching hybrids with the selected female parent separately for height and base diameter, the characters which with non-branching habit determine the quality of jute fibers, and no evidence of a significant difference was found. The hybrid, however, performed slightly better than the control, suggesting that further selections may yield better strains than the existing standard varieties of jute.

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REDS HATCH CHICKENS A LA RUSSE

Hens From USSR Injected With Blood From U. S. Roosters Are Reported to Lay Some Odd Eggs

A series of controversial reports by Soviet geneticists has been topped by a claim that predictable genetic transformations can be induced at will in some higher organisms without even using desoxyribonucleic acid, the "hereditary messenger."

This latest report, presented at the USSR Industrial and Scientific Exhibit in Paris, was authored by Professor Khilia Kushner of the Genetics Institute of the Soviet Academy of Sciences. He concludes that transformations are not necessarily influenced by one "heredosubstance." They can be altered by any factor which has the capacity of modifying the metabolism of the recipient in such a way that it modifies the metabolism of its reproductive cells. American geneticists and biochemists, however, have expressed doubts.

Blood Will Tell

The organisms used by Dr. Kushner were two varieties of chicken: snow-white Russian fowl, which had bred true for at least five generations, and dark New Hampshires. In the first stage of the experiment, he injected doses of 5 ml of blood from the New Hampshires twice a week into a vein under the wing and into pectoral muscles of the Russian chickens. After 150 to 160 ml had thus been injected, reports Dr. Kushner, the white chickens, even though mated with white roosters, started hatching some chicks with spottily distributed dark feathers. By the third generation, the percentage of "transformed" fowl had reached 40, with some of the animals turning out completely black or ash gray. The new traits were transmitted to further generations, both by males and females, while control studies of Russian chickens inoculated with blood from the same breed resulted in no change at all, reported the Soviet geneticist.

In the second stage of the experiment, he attempted to determine which component of the blood was responsible for the genetic changes—and while he admits failure so far, he has come to the rather surprising conclusion that DNA was not it. He reports that in spite of centrifuging New Hampshires' blood, and extracting from it all cellular elements and DNA before injecting it, the same morphological changes appeared in the progeny. Similar experiments with rabbits, he says, resulted in changes of fur color. Again the changes were the same whether whole rabbit blood or only plasma was used, according to Dr. Kushner.

Genetics vs. Politics

The significance of such startling results, however, is tempered by Western distrust of Soviet genetics, which were adapted to political needs during the Stalin era by T. D. Lysenko—now back at the helm of the Soviet Genetics Institute. Lysenko has long been supporting the point (compatible with Marxist theories) that the nature of an organism could be altered by a variety of environmental

(Continued on Page 296)

DELETERIOUS EFFECTS OF A GENE CAUSING EXCESSIVE PIGMENTATION

In The Platyfish

PAMELA A. MAC INTYRE*

THE dominant, sex-linked *Fu* (*fuliginosus*) gene, which controls a sooty black coloring by macromelanophores over most of the body, appeared as a mutation in a platyfish, *Xiphophorus maculatus,* bred by German aquarists. It was first described by Kosswig[5] in 1938. Most *Fu* males and a few females develop melanomas—and erythrophoromas, if red pigment is present[1]—on various parts of the body, but particularly on the anal fin, thus interfering with reproduction (Figure 15). In xiphophorin fishes, melanomas usually occur only in certain interspecific hybrid combinations[3]. *Fu* fish also show lowered vitality and viability.

Kosswig[5] described the *Fu* gene as semi-lethal; he assumed that homozygous *Fu* fish existed but was never able to find a fertile homozygote. Öktay[6], in a series of observations in this stock, stated that homozygous *Fu* fish never occurred due to incompatibility of *Fu* ova and *Fu* sperm. She found no evidence for differential lethality during embryonic or post-embryonic stages.

In 1950 some *Fu* fish were obtained by this laboratory from Kosswig. These were outcrossed to other platyfish stocks and then inbred. Thus, this laboratory's stock is similar to Kosswig's probably only in the presence of the *Fu* gene. In the strain reported here, three sex chromosomes are present, corresponding in strength to the W, X, and Y chromosomes described by Gordon[2] in other *X. maculatus* strains; the combinations WY, WX, and XX are females, XY and YY males. *Fu* is found only on the Y chromosome. Gordon and Baker[4] presented some data indicating that when homozygous the *Fu* gene causes death between birth and 35 days of age.

Results and Discussion

Further studies made in this laboratory now indicate that, in different *Fu* crosses, different percentages of *Fu* fish reach birth. In the usual type of mating, $WY_{Fu} \times XY_{Fu}$, the ratio of *Fu* : + at birth ranges from 2 *Fu* : 1 + to 3 *Fu* : 1 +. There are few deaths until after sexual differentiation. Deficiencies of *Fu* fish (from the expected 2 *Fu* males : 1 *Fu* female : 1 + female) are generally of *Fu* males. It has customarily been assumed that the deficient males are the homozygotes, but these can be distinguished from heterozygotes only by matings. Many *Fu* males are sterile; those that have bred have always proved to be heterozygotes, but recently one exception was found. When mated to a WY_{Fu} female, this fish produced 60 *Fu* (22 females, 31 males, 7 sex unknown) and no + young. When mated to an XX female of another strain, 37 *Fu* males and no + were produced. A series of other matings of *Fu* males (including two siblings of the exceptional fish) to XX females always resulted in ½ + females and ½ *Fu* males, indicating the XY_{Fu} composition of the male parents. It is possible that the exceptional fish was XY_{Fu} and that either it produced no X sperm or embryos containing its X chromosome were inviable. It seems more likely, however, that by chance a rare fertile homozygous male was detected and that such fish had not been detected before because of (1) a quantitative difference in fertility between homozygotes and heterozygotes (the former probably likely to become sterile earlier) and (2) perhaps, in some crosses, a differential pre-natal lethality of homozygotes or non-union of *Fu* sperm and ova.

Fu females show little variation in the

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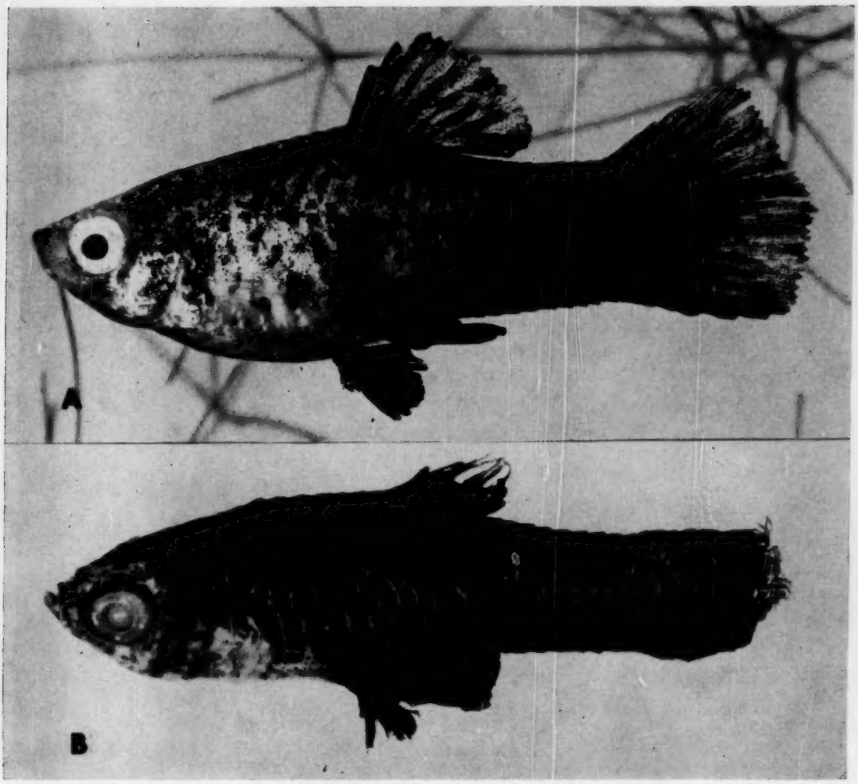

PIGMENTED PLATYFISH

Figure 15

A—A *Fu* (*fuliginosus*) male platyfish, *Xiphophorus maculatus,* commonly called the Fury variety, with an abnormal accumulation of black pigment cells on its gonopodium (the modified anal fin used in reproduction). This represents an early stage in melanoma development. *B*—A *Fu* male with a large melanoma on its gonopodium.

amount of black pigmentation present. In males considerable variation exists, but there is no correlation between darkness and homozygosity or heterozygosity of the *Fu* gene, known heterozygotes often being quite dark and vice versa. Of the 31 presumably homozygotic males produced by the mating of the exceptional $Y_{Fu}Y_{Fu}$ male to a WY_{Fu} female. 12 were quite light.

The exceptional homozygous male was darkly pigmented. It was preserved for histological examination while in a vigorous condition at one year of age. The testis was normal. It had a large melanoma and erythrophoroma on the body, but the growth of pigment cells on the gonopodium (the modified anal fin used in reproduction) was only slightly melanotic, not tumorous. *Fu* males usually have gonopodial melanomas at this age. The difference in fertility between homozygotes and heterozygotes may result from a tendency of the former to earlier hyperplasia of pigment cells on the gonopodium, the fish in question being unusual in this respect.

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✦

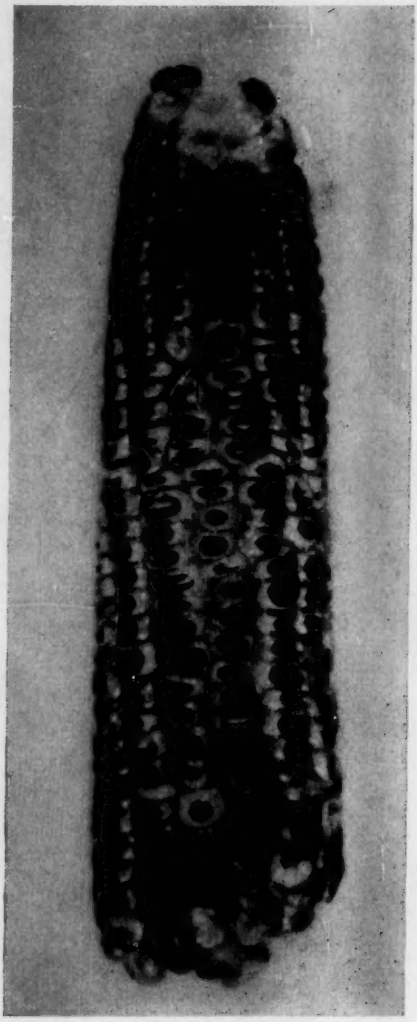
THE DEMONSTRATION OF WAXY IN MAIZE

A. R. A. Noel

IN THE course of building up a collection of maize cobs for the teaching of grain factor inheritance, Mendelian ratios and linkage, the problem arose as to the most satisfactory method of demonstrating the factor for waxy endosperm. Unless invariably linked to shrunken this factor is difficult to detect externally, particularly in the presence of colored pericarp or endosperm. Any attempt to expose the endosperm on the ripe cob usually results in detachment of many of the grains. The following technique was therefore devised to demonstrate the distribution of waxy among the grains while still on the cob.

A syrupy solution of "Perspex" in chloroform is poured onto a mature, dry cob. The solution dries very rapidly, and by rotating the cob in the hand as the solution is poured on, a thick coating of plastic can be built up between the grains. The cob is then suspended in an oven at 70° C. for two days. At the end of this time the grains are firmly attached to the axis and any lateral movement is prevented by the plastic matrix. The cob may now be held against an electric sander, fitted with a fine grade abrasive, and ground down until the endosperm is exposed in each grain. On dipping the cob into dilute iodine solution the waxy grains stain reddish brown while the normal starchy endosperm becomes blue-black. The cob may be stored dry, or preferably in 70 percent alcohol, and the iodine test repeated as often as desired.

By using this method a very striking contrast can be obtained between waxy and non-waxy grains and the segregation is readily determined. Besides providing a good demonstration of the inheritance of physiological variation, the factor waxy is particularly useful in teaching the measurement of linkage.



WAXY CORN

Figure 16

This cob of corn shows the striking differences between waxy and non-waxy kernels. See text for details.

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A RED LEAF MARKING IN WHITE CLOVER

ARNE W. HOVIN AND PRYCE B. GIBSON*

SEVERAL hereditary leaf markings have been reported in white clover, *Trifolium repens* L.[1,2,3]. White clover plants with a red-purple leaf marking were found in Georgia and South Carolina pastures by Mr. Paul Tabor and the junior author, respectively. The marking is V-shaped on young leaflets (Figure 17), but as the leaflets become older, the reddish coloration spreads within the V, extending frequently to the proximal and distal ends of the midrib. Anthocyanin is assumed to be the pigment. This rare marking, which has the same color as red flecking, red midrib and red leaves, is expressed best in cool weather. Its inheritance is reported below.

WHITE CLOVER LEAF MARKING
Figure 17

On the left is shown a green leaflet with no marking and on the right a leaflet with a red marking superimposed on a white marking.

Materials and Methods

One of the parent plants used in this study had white leaf markings and the other had white as well as red markings. The white marking was believed to be V^b according to the nomenclature by Brewbaker and Carnahan[2]. The resulting F_1's from this cross were assumed to be either homozygous V^bV^b or heterozygous V^bv with respect to the white marking. The F_1 plants with red leaf marking (presumably heterozygous red) were selected and crossed with green, self-incompatible testers carrying no leaf markings. The F_2 populations were obtained by intercrossing self-incompatible F_1 plants. Mature seeds that had been harvested from individual plants were scarified and pregerminated on moistened filter paper in Petri dishes before planting in flats filled with sterilized soil. The plants were studied in the greenhouse.

Results

Testcrosses and F_2 populations segregated red to green, 1 : 1 and 3 : 1 (Table I), respectively, indicating that the red leaf marking character was simply inherited and dominant to the allele for no red pigment. Furthermore, the data in Table I suggested that this red leaf marking gene was closely linked to the 11 *V*-alleles described by other workers[1,2,3]. The symbol $V^r{}_2$ is proposed for the gene controlling the red marking studied here. The testcrosses demonstrated that the F_1 plants reported in Table I carried the red $V^r{}_2$ and white V^b alleles in the coupling phase.

Three additional sets of testcrosses were made with a different tester in each set and heterozygous F_1 plants with $V^bV^r{}_2$ in coupling. The two non-parental plants (Table II) were green with white markings only. Two questionable plants identified as "red marking only" were later observed to have white markings also. The calculated crossover value was 0.1 percent, but the limited population size could not rule out the possibility of mutations at either the $V^r{}_2$ or the *V* locus. The fact that plants with red markings always had white markings suggested that the $V^r{}_2$ allele was

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closely linked to and possibly under control of the *V* locus. Plants homozygous for the red marking were not obtained from these studies.

Summary

A new red leaf marking in white clover was found to be controlled by a single dominant gene designated V^r_s. A crossover value of 0.1 percent was obtained between the V^r_s and the *V* locus.

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TABLE II. Segregations in three testcross progenies of white clover and percentage of crossover between V^r_2 and V^b

Tester	No. of crosses	Observed phenotypes: Parental	Observed phenotypes: Non-parental	Crossover (%)
58–65	7	1044	0	0
58–66	11	1159	1	.09
58–67	10	869	1	.11
Total	28	3072	2	.10

TABLE I. Progenies from crossing F_1 plants of white clover with red and white leaf markings to green testers without markings and from F_2 populations

F_1 parent (s)	Generation studied	No. of crosses	Observed phenotypes: Red, white	Green, white	Green, none	Expected ratio	χ^2	P
Heterozygous red, heterozygous white	Testcross	7	122	0	134	1:1	.56	.20–.50
	F_2	2	131	0	31	3:1	2.97	.05–.10
Heterozygous red, homozygous white	Testcrosses	2	13	9	0	1:1	.73	.20–.50
	F_2	1	42	14	0	3:1	.00	1.00
Heterozygous red, one homozygous white, one heterozygous	F_2	7	486	159	0	3:1	.04	.50–.95

Reds Hatch Chickens a La Russe

(Continued from Page 291)

influences—an idea generally and wholeheartedly rejected by Western geneticists. They have found that mutations occur only when genes are physically altered, as by direct action of a chemical or of radiation.

If the results are ever confirmed, it could lead to a drastic revision of the present concepts on the mechanism of heredity.

But one U.S. scientist, who attempted to duplicate the first stage of the Kushner experiment with chickens, says he has succeeded in producing only "more flocks of white chickens." Many others believe that the Soviet experiment may have slipped up somewhere along the way, as in the selection of a "pure" breed of fowl. As one expert put it: "Was anybody watching the chickens all the time?"

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THE POSITION OF LUXOID IN LINKAGE GROUP II OF THE MOUSE

MARGARET C. GREEN*

THE MUTANT gene luxoid (*lu*) has been described by Green[9] and in considerably more detail by Forsthoefel[6,7]. Briefly, *lu* causes preaxial abnormalities of the limbs resulting in preaxial polydactyly of the forefeet and tibial hemimelia in homozygotes, and preaxial polydactyly of the hind feet in heterozygotes. Penetrance in the heterozygote is limited and dependent on the genetic background. This paper reports the results of linkage tests which show *lu* to be located in linkage group II on the opposite side of short ear (*se*) from ducky (*du*).

Results

Linkage of *lu* with *se* was discovered in a repulsion intercross between mice of genotype *aa b+ pc*^{ch}*/++ lu++/ +dse*. The joint segregation of *lu*, *d*, and *se* is given in Table I, cross 1. The classification of *d* may not be completely accurate because of the other color genes segregating in the cross and because of the occasional tendency of *d* to be expressed in the juvenile coat of heterozygotes. Since *d* and *se* are very closely linked, with a recombination of about 0.15 percent[8], they may be, for most practical purposes, regarded as a single locus, in this case identified by *se*. The maximum likelihood estimate of the recombination frequency, *p*, between *lu* and *se*, calculated with the aid of Finney's tables[4] (Table 3b, $p = .15$) is 14.06 ± 4.49 percent.

From this cross it was possible to recover the *ludse* chromosome and to make up intercross matings in coupling (Table I, cross 2). The severe shortage of both *sese* ($\chi^2 = 17.33$) and *lulu* ($\chi^2 = 14.77$) among the offspring makes the maximum likelihood estimate based on the assumption of equal viability of all classes unreliable. Bailey[1] has shown that the product method[5] gives an accurate estimate of recombination when the viability of both recessives is reduced. The estimate by this method in the coupling intercross is 19.93 percent. The standard error calculated by the formula given by Bailey[1] is 3.69 percent.

Homozygous *lulu* males are completely sterile and females almost completely so. It was therefore not possible

TABLE I. Results of intercrosses in repulsion and coupling for *lu*, *d*, and *se*: *lu* is treated as a recessive. Numbers in parentheses indicate number of mice which died before they could be classified for *se*. They are included in the class totals assuming they were not recombinants between *d* and *se*

Cross	Parents	Phenotype of offspring							% Recombination
		+++	+dse	++se	+d+	lu++	ludse	Total	lu–se
1	lu + + / + d se × lu + + / + d se	244	115	1	2	105	2	469	14.06±4.49
2	+ + + / lu d se × + + + / lu d se	169(7)	11(2)	—	—	13(1)	15(1)	208	19.93±3.69

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work was supported in part by research grants NSF G–7023 from the National Science Foundation and ACS E–162 from the American Cancer Society. The author is grateful to Mrs. Hope O. Sweet for invaluable assistance in managing the mouse colony. This paper is dedicated with affection and respect to Professor L. C. Dunn on the occasion of his retirement as Professor of Zoology at Columbia University.

to make the usual kind of backcross treating *lu* as a recessive. Instead it was treated as a dominant with incomplete penetrance. On the genetic background of the C57BL/10JGn (here called C57) inbred strain, the penetrance of *lu*+ is close to 90 percent. Mice which were the product of two to four generations of crossing to C57 were used to make up the matings listed as crosses 3, 4, 5, and 6 in Table II. Recombination frequencies and penetrance were estimated by the maximum likelihood formulas given by Bailey[2].

The estimates of recombination between *lu* and *dse* are higher in repulsion (crosses 4 and 6, *lu* considered as a dominant) than in coupling (crosses 3 and 5) and in males (crosses 5 and 6) than in females (crosses 3 and 4). The standard errors of these estimates are large, however, and neither of the differences is significant. The combined estimate of recombination in crosses 1 through 6, calculated by weighting each estimate by the reciprocal of its variance, is 17.15 ± 2.11 percent.

The estimates of penetrance of *lu* vary widely in crosses 3 to 6. With males and females combined, the estimate for coupling (crosses 3 and 5) is 48.59 ± 5.25 percent and for repulsion (crosses 4 and 6) is 85.35 ± 7.24 percent. This difference is highly significant ($t = 4.11$, $P < .01$), reflecting, no doubt, a difference in frequency of C57 genes in the two crosses. There was more selection of modifiers favoring penetrance of *lu* in the preparatory crosses for the repulsion matings than for coupling. With coupling and repulsion combined, the estimate of penetrance for offspring of *lu*+ *dse*/++ females (crosses 3 and 4) is 69.57 ± 5.23 percent, and for offspring of ++ *dse*/*dse* females (crosses 5 and 6) is

TABLE II. Results of linkage crosses with *lu*, *d*, *se*, and *du*; *lu* is treated as a dominant with reduced penetrance Numbers in parentheses indicate number of mice which died before they could be classified for *se*. They are included in the class totals assuming they were not recombinants between *d* and *se*

Cross	Parents ♀	Parents ♂	Phenotype of offspring +++	+*dse*	*lu*++	*ludse*	Total	% Recombination *lu-dse*	% Penetrance of *lu*
3	*lu* + + / + *d se*	+ *d se* / + *d se*	32(4)	57(6)	29(3)	4(1)	122	12.12± 5.34	54.10± 7.14
4	+ + + / *lu d se*	+ *d se* / + *d se*	45	16*	8	42	111	17.25± 4.69	87.51± 7.69
5	+ *d se* / + *d se*	*lu* + + / + *d se*	43	49(5)	21	5	118	22.01± 7.69	42.07± 7.18
6	+ *d se* / + *d se*	+ + + / *lu d se*	9	3	2	3	17	26.67±15.73	68.18±21.66
			++	+*se*	*du*+	*duse*	Total	*du-se*	χ^2(*du*)†
7	+ + / *du se*	+ *se* / *du se*	115	79	9	33	236	21.26± 4.71	6.53
8	+ *se* / *du* +	+ *se* / *du se*	26	35	7	2	70	22.59± 8.65	5.50
9	+ *se* / *du se*	+ *se* / *du* +	34	49	21	3	107	15.04± 7.00	0.38

* Includes one ++*se* mouse.
† χ^2 for segregation of *du*.

45.04 ± 7.30 percent. This difference is also highly significant ($t = 2.73$, $P < .01$). Forsthoefel[6] found that, in a strain formed by crossing *lu* into C57BL/10JGn for many generations, penetrance was higher when the female parent was ++ than when it was *lu*+, an opposite result from the present one if only the *lu* locus is considered. In the present case the female parents in the reciprocal crosses differ also at the *d* and *se* loci and possibly at other loci in linkage group II. In addition, the reciprocal crosses may differ at other unlinked loci, since matings were in most cases not made up reciprocally using littermates. Therefore, the present results do not support any conclusion about the effect of the parental genotype with respect to *lu* on the penetrance of *lu* in the offspring.

The linkage of ducky (*du*) with *d* and *se* was reported by Snell[11]. Because of the possibility of misclassification for both *du* and *d* the amount of recombination was left considerably in doubt. In 1956 we obtained some mice of the ducky stock from Mrs. Elizabeth Woodworth, who maintained the stock for Dr. Snell, with the intention of setting up crosses to determine the order of *lu*, *du*, *d*, and *se*. The stock was maintained with *du* and *se* in repulsion. We were fortunate enough to recover a chromosome with *du* and *se* in coupling and from it produced the single backcrosses listed as crosses 7, 8, and 9 in Table II. It was necessary to use single backcrosses rather than double backcrosses because of the very low fertility of homozygous *dudu* mice. There is a significant deficiency of *dudu* in crosses 7 and 8, due almost certainly to reduced viability of this class. Estimates of recombination should not be affected by reduced viability at one locus, however, and the good agreement between coupling and repulsion in females (crosses 7 and 8) confirms this expectation. The estimates of recombination calculated with the aid of Finney's tables[4] (Table 2, $p = .20$) are higher for females than for males, but the small number of offspring of male double heterozygotes makes the standard error large, and the difference is not significant. The combined estimate from crosses 7, 8, and 9 is 19.87 ± 3.56 percent.

Because of the infertility of *lulu* and *dudu*, it was decided to make the 4-point cross as follows: *ludse*+/+++*du* × +*dse*+/+*dse*+. The polydactylous (*lu*+) offspring were then progeny tested for *du* by mating to known *du*+ mice. Preliminary to making the 4-point cross it was necessary to cross *du* into C57 several times in order to obtain sufficiently high penetrance of *lu*. The 4-point crosses were in fact those listed as crosses 4 and 6, Table II. The polydactylous offspring classified for *du* are given in Table III. One animal classified as not *du* produced only 11 offspring but all others produced at least 12 and most produced 14 or more. Although there is a deficiency of the parental type, *ludse*+, it is clear that, of the recombination classes, *lu*+++ is by far the least frequent and is therefore the double crossover class. The order is thus *lu*-(*dse*)-*du*.

TABLE III. Frequency of parental and recombinant chromosomes among polydactylous (*lu*+) offspring of linkage crosses. Presence or absence of *du* was determined by progeny test

Cross	Parents	*ludse*+	*lu*++*du*	*ludse du*	*lu*+++	Total	
4, 6	$\frac{lu\ d\ se\ +}{+\ +\ +\ du}$ + $\frac{+\ d\ se\ +}{+\ d\ se\ +}$	11	6	12	1	30	
		lu+	*ludu*			Total	% Recombination *lu-du*
10	$\frac{lu\ +}{+\ du}$ + $\frac{+\ +}{+\ +}$	27	14			41	34.15±7.41

Simultaneously with the 4-point cross, a similar cross using *du* and *lu* alone was made, and 41 of the polydactylous offspring were progeny tested for *du* as above. The results are shown as cross 10, Table III. Fourteen of the 41 were recombinants, giving an estimate of $p = 34.15 \pm 7.41$ percent, in good agreement with expectation if *lu* and *du* are on opposite sides of *dse*.

One female from cross 4 was normal footed, intense colored, and short eared. She therefore carried a crossover between *d* and *se*, and it was anticipated that she would be found either to carry both *lu* and *du* (indicating the order *lu se d du*) or to carry neither (indicating the order *lu d se du*). When mated to a *ludsedu*/+*dse*+ male she produced both polydactylous and *dudu* but no *lulu* offspring among 41, and when mated to a *du*+ male she produced one *dudu* but no polydactylous offspring among 10. Her genotype was thus probably ++*sedu*/+*dse*+. The results of the test mating to the *ludsedu*/+*dse*+ show that her genotype was probably not +*dsedu*/++*se*+. The mating produced (ignoring classification for *lu*) 20 *dse*+, 16 +*se*+, 2 *dsedu*, and 3 +*sedu* offspring, indicating that *d* and *du* were more probably in repulsion than in coupling. This makes it unlikely that the exceptional female received a mutation from *d* to + from her father. The possibility that she received a mutation from + to *se* from her mother cannot be ruled out. If her genotype was not due to mutation, however, she must have received a chromosome carrying a double crossover from her mother. The second crossover could have occurred on either side of *dse*, and therefore provides no information on the order of *d* and *se* with respect to *lu* and *du*.

Discussion

Under ordinary assumptions about interference, the probability that a chromosome carrying a crossover between two closely linked genes will also carry another crossover within about 20 units on either side is rather low. A rough estimate of the probability may be obtained. Carter[3], using Slizynski's data on chiasma frequency in male mice, has calculated that the standard deviation of the distance between one chiasma and the next is 0.3095 times the mean distance. Assuming that the mean distance between chiasmata corresponds to 50 centimorgans (cM), the probability of a second chiasma within 20cM of the first is approximately the probability corresponding to a normal deviate greater than 1.94 ($=30/50 \div 0.3095$). This probability is 0.026, and the probability for a second chiasma on either side is therefore 0.052. Since crossovers occur in only two of the four chromatids at a chiasma, the probability for a second crossover on either side is $0.052/2 = 0.026$. While this probability is not prohibitively low, it nevertheless suggests that in the mouse, as well as in microorganisms (see Pritchard[10]), interference over very short distances may be much less that over greater distances. Further crosses will be necessary to determine whether such double crossovers occur with greater than expected frequency and to rule out mutation as an explanation. Such an experiment will have to await the availability of more satisfactory marker genes in linkage group II.

Summary

The arrangement of four loci in linkage group II has been found to be

lu 17 (*dse*) 20 *du*

One crossover between *d* and *se* was found. Under the assumption that it was indeed a crossover, not a mutation, it proved to be a double crossover, suggesting the possibility of low or negative interference over very short distances.

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A STUDY OF SPOTTING PATTERNS IN THE LEOPARD FROG

I. Effect of Gene Dosage

JOHN DAVISON*

THIS paper is the first of a series dealing with the genetic and developmental mechanisms responsible for the dorsal spotting patterns in the leopard frog, *Rana pipiens*. It is remarkable how little is known concerning the most conspicuous feature of the animal. Fortunately, a genetic tool is available in the form of the burnsi gene. Moore[7] first described the locus as a semi-dominant, with homozygous (*BB*) animals lacking spots entirely, and heterozygotes (*Bb*) retaining spots on the appendages, but not the back. The recessive (*bb*) wild-type animal is, of course, spotted on both back and legs.

By means of induced and spontaneous triploidy it is possible to obtain additional gene dosages to give a balanced series of dosages from zero to one, as follows: 0 (*bb, bbb*), ⅓ (*Bbb*), ½ (*Bb*), ⅔ (*BBb*) and 1 (*BB, BBB*). Preliminary experiments with diploid heterozygotes indicated that low temperature during metamorphosis promotes spotting, while high temperature inhibits spotting. The specific effects of temperature on the expressivity of the burnsi locus will be discussed in a later paper. The present study concerns effects of gene dosage under standard temperature conditions (approximately 24° C.).

Materials and Methods

All frogs were obtained from professional dealers in Wisconsin and Minnesota, and maintained at about 5° C. until ready for use. Ovulation and fertilization were carried out according to Rugh[8], and triploidy was induced by methods similar to those of Briggs[1] (heat treatment at 37° C. for four minutes beginning 16 minutes after fertilization). The larvae were reared on chopped boiled spinach and lettuce. Following metamorphosis, a few of the animals were reared to adults on a diet primarily of crickets.

Two methods were used to determine the degree of polyploidy in control and experimental animals. Chromosome counts were made in tail fin squashes according to Hungerford and DiBerardino[5] (treatment of the excised tail tip for 45 minutes in 38 percent Modified Niu-Twitty Solution, followed by fixation and light staining in acetocarmine). The preparation was then squashed and sealed and the chromosomes counted in intact metaphase plates (phase contrast). *Rana pipiens* has a haploid number of 13 chromosomes[10], so diploids, triploids and tetraploids have 26, 39 and 52 chromosomes, respectively. The frog is among those species with a single large nucleolus for each chromosome set[3]. Maximum nucleolar number (the nucleoli have a tendency to fuse in resting nuclei) is thus a measure of the degree of ploidy. Tail tips were excised shortly after hatching, fixed, stained with hematoxylin and whole mounted. Nucleolar numbers were recorded in the epidermal cell nuclei, which are clearly larger in triploids. By preparing a squash from the regenerated tip, the two methods were found to be equivalent, in agreement with earlier studies[4].

Both heat treated and control animals were screened for possible polyploids. Suspected polyploid larvae can be identified shortly after hatching by epidermal cell size, and in later stages by the greater spacing and size of the yel-

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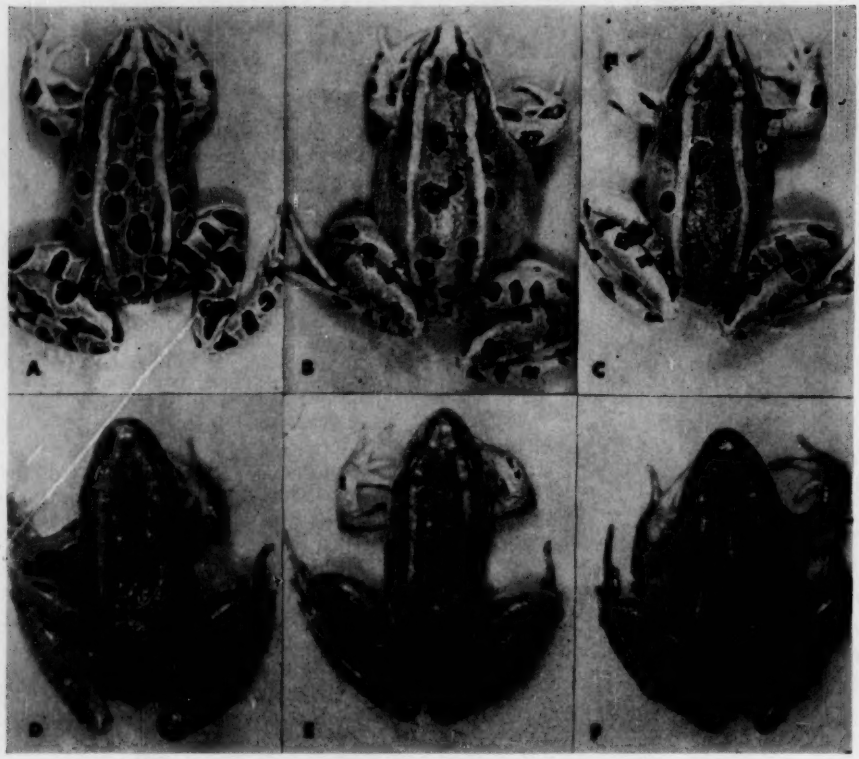


BURNSI PHENOTYPES

Figure 18

Typical adult phenotypes carrying the burnsi gene in dosages from zero to one. *A*—Adult wild-type *R. pipiens*. This animal happens to be a triploid (*bbb*), but the pattern is indistinguishable from a diploid recessive. *B*—Tetraploid adult of genetic constitution *Bbbb*, obtained from the cross *bb* ♀ × *Bb* ♂. *C*—Triploid adult of genetic constitution *Bbb*, obtained from the same cross. *D*—Adult diploid heterozygote (*Bb*) of the type used as parental stock. *E*—Triploid type *BBb*. *F*—Homozygote type *BB* or *BBB*.

low chromatophores. Induced triploidy ranged from 30 to 80 percent. Although only a single spontaneous triploid was discovered among the untreated eggs in this study, the author has on other occasions found higher percentages[2]. Certainly in the frog, and other forms with an unreduced egg gamete, spontaneous triploidy is possible and should be checked routinely.

Three basic crosses were made. (1) Homozygous recessive (wild-type) females were crossed with heterozygous burnsi males (*bb* ♀ × *Bb* ♂). (2) The reciprocal cross was made (*Bb* ♀ × *bb* ♂). (3) Heterozygotes were crossed (*Bb* ♀ × *Bb* ♂). Typically, sperm was obtained from two males. All burnsi parents were of the type with strong leg spots (Type D, Figure 18).

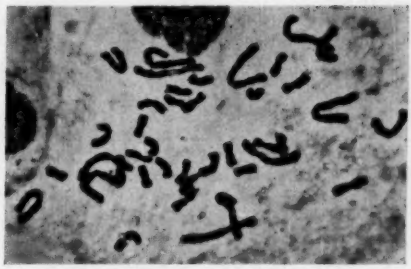
Results

Table I summarizes the results of the three crosses. In the first (*bb* ♀ × *Bb* ♂), suppression of the second meiotic division should produce only two types of triploids, *bbb* wild-type and *Bbb* burnsi animals. A single spontaneous triploid was found among the untreated larvae, and a single tetraploid

(52 chromosomes) among the treated larvae. The triploid burnsi animals clearly have more dorsal spotting than the diploid heterozygotes and accordingly are ascribed the genetic constitution *Bbb*, as would be expected from meiotic suppression. The single tetraploid shows even less inhibitory influence of the burnsi locus, indicating a genotype of *Bbbb*. There are a variety of assumptions one can make to explain the cytological origin of the tetraploid genotype. One possibility is the fusion of two sperm (dispermy), one dominant and one recessive, with the unreduced egg gamete to yield the *Bbbb* fusion nucleus. In order of increasing burnsi dosage, the four types which were recovered are pictured in Figure 18 (*A, bbb* or *bb* wild-type; *B, Bbbb* tetraploid; *C, Bbb* triploid; and *D, Bb* diploid).

In the second cross (*Bb* ♀ × *bb* ♂), three triploid classes can be expected. *BBb* and *bbb* represent the non-cross-over classes, while *Bbb* animals result from the fusion of the recessive sperm nucleus with the cross-over female gamete *Bb*. It is surprising to notice that the cross-over class is by far the most frequent (Table I).

The third cross yielded the expected three diploid classes (*bb, Bb* and *BB*) and the four triploid classes (*bbb, Bbb, BBb* and *BBB*), each of which can be discriminated phenotypically when compared with the diploids. In the absence of crossing-over, the triploid classes would occur with equal frequency. However, as in the last cross, an apparent high frequency of crossing-over leads to a reduction, in this case, of the homozygous classes (*bbb, BBB*). Typical examples of each of the phenotypes are illustrated in Figure 18.



METAPHASE OF TRIPLOID BURNSI
Figure 19
Triploid metaphase plate from pre-treated tail tip.

Discussion

It is not the primary purpose of this paper to evaluate frequency of crossing-over, since the number of animals involved is too small to permit an accurate estimate. However, the suggested high frequencies are not surprising when these findings are compared with those of Lindsley *et al.*[6] They employed the technique of induced triploidy to estimate the cross-over frequency of three recessive unlinked genes in the axolotl. By crossing heterozygous females with homozygous recessive males, cross-over frequency can be calculated from the number of recessives among the triploid larvae produced. They observed frequencies of 0.688, 0.694 and 0.724, respectively, for the characters sex (the female axolotl is heterogametic), color and fluid imbalance. For a simple recessive, the frequency of crossing-over is given by one minus twice the frequency of the recessive class. In the comparable burnsi cross (*Bb* ♀ × *bb* ♂), one can recognize the cross-over class directly (*Bbb*) and calculate a frequency of $^{13}/_{15}$. When crossing heterozygotes with heterozygotes, the frequency is given by one minus twice the sum of the homozygous classes or $1-(^{6}/_{19})$. The average for these cal-

TABLE I. Summary of diploid and polyploid progeny from crosses between burnsi and wild-type parents

Cross	Controls			Treated			
bb × *Bb* ♀ ♂	*bb* 12	*Bb* 16	*Bbb** 1	*bbb* 6	*Bbb* 11	*Bbbb* 1	
Bb × *bb* ♀ ♂	*bb* 18	*Bb* 18		*bbb* 1	*BBb* 1	*Bbb* 13	
Bb × *Bb* ♀ ♂	*bb* 7	*Bb* 30	*BB* 13	*bbb* 2	*Bbb* 9	*BBb* 7	*BBB* 1

* Spontaneous triploid.

culated frequencies is 0.78, in the same range as the values reported for the axolotl. Lindsley *et al.* concluded that either the axolotl genes were all located near chromosome ends or, alternatively, that the centromeres separated randomly during the first meiotic division. Either assumption would account for frequencies near ⅔. It is interesting to note that all four of these genes have been found to have apparent cross-over frequencies above the theoretical limiting value of 0.67. The possibility cannot yet be ruled out that in the frog and axolotl, it is the sister strands which separate in the first meiotic division, in contrast with typical meiosis.

With respect to the major objective of the study, it is clear that the burnsi locus is incompletely dominant, progressively inhibiting dorsal spotting as the dosage is increased from ¼ to 1. Back spotting is inhibited at dose levels between ⅓ and ½, while leg spotting is extinguished between ⅔ and 1 (Figure 18). It should be noted that all the burnsi larvae, including the tetraploid, failed to develop the black spots characteristic of wild-type recessives, indicating that tadpole spotting can be completely inhibited by dosages as low as ¼. Thus tadpole skin, adult back skin and adult leg skin, in that order, demonstrate decreasing sensitivity to the inhibitory effect of the burnsi locus.

Recently Volpe[9] has contended that modifier genes greatly alter the expression of the burnsi locus. Crossing a heterozygous male and a wild-type female (the same as the first cross reported here), he recovered burnsi progeny, some of which had a few back spots. These closely resemble the induced and spontaneous triploids (*Bbb*) described in this paper. Incidentally, this is the only triploid burnsi type to be expected from a cross involving a wild-type female. Since Volpe does not mention any analysis for triploidy, the possibility must be considered that some or all of his back-spotted burnsi animals are spontaneous triploids of the genotype *Bbb*. In any event, this type was not observed among known diploids in the present study.

To avoid future confusion concerning this point, it should be mentioned that it is possible to obtain diploid heterozygotes with a few back spots under the special conditions of growth and metamorphosis at low temperatures (18° C. or lower)[2]. Differences in temperature during natural metamorphosis may account for much of the variation observed among the burnsi heterozygotes supplied by the dealers.

Summary

By means of induced and spontaneous polyploidy, frogs were produced carrying the burnsi locus in balanced dosages of 0, ¼, ⅓, ½, ⅔ and 1. The locus is clearly incompletely dominant.

The phenotypes indicate extinction of back spots at a dosage between ⅓ and ½, while leg spotting is inhibited between ⅔ and 1. Larval spotting is prevented by dosages as low as ¼.

Analysis of triploid progeny from heterozygous females indicates a high apparent cross-over frequency for the burnsi locus comparable with that observed in similar studies with the axolotl[6].

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